

This is a repository copy of *Sodium homeostasis in the tumour microenvironment*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/148601/>

Version: Accepted Version

---

**Article:**

Leslie, Theresa Katharina, James, Andrew David, Zaccagna, Fulvio et al. (8 more authors) (2019) Sodium homeostasis in the tumour microenvironment. BBA - Reviews on Cancer. pp. 1-16.

<https://doi.org/10.1016/j.bbcan.2019.07.001>

---

**Reuse**

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: <https://creativecommons.org/licenses/>

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

## **Sodium homeostasis in the tumour microenvironment**

Theresa K. Leslie<sup>1,2</sup>, Andrew D. James<sup>1,2</sup>, Fulvio Zaccagna<sup>3</sup>, James T. Grist<sup>3</sup>, Surrin Deen<sup>3</sup>,  
Aneurin Kennerley<sup>2,4</sup>, Frank Riemer<sup>3</sup>, Joshua D. Kaggie<sup>3</sup>, Ferdia A. Gallagher<sup>3</sup>, Fiona J.  
Gilbert<sup>3</sup> and William J. Brackenbury<sup>1,2\*</sup>

<sup>1</sup>Department of Biology, University of York, Heslington, York, YO10 5DD, UK

<sup>2</sup>York Biomedical Research Institute, University of York, Heslington, York, YO10 5DD, UK

<sup>3</sup>Department of Radiology, University of Cambridge, Cambridge Biomedical Campus  
Cambridge, CB2 0QQ, UK

<sup>4</sup>Department of Chemistry, University of York, Heslington, York, YO10 5DD, UK

\*Corresponding author:

Dr William J. Brackenbury, Department of Biology and York Biomedical Research Institute,  
University of York, Wentworth Way, Heslington, York YO10 5DD, UK

Email: [william.brackenbury@york.ac.uk](mailto:william.brackenbury@york.ac.uk)

Tel: +44 1904 328284

Fax: +44 1904 328505

**Keywords:** Channels / Microenvironment / MRI / Sodium / Transporters / Tumours

## **Abstract**

The concentration of sodium ions ( $\text{Na}^+$ ) is raised in solid tumours and can be measured at the cellular, tissue and patient levels. At the cellular level, the  $\text{Na}^+$  gradient across the membrane powers the transport of  $\text{H}^+$  ions and essential nutrients for normal activity. The maintenance of the  $\text{Na}^+$  gradient requires a large proportion of the cell's ATP.  $\text{Na}^+$  is a major contributor to the osmolarity of the tumour microenvironment, which affects cell volume and metabolism as well as immune function. Here, we review evidence indicating that  $\text{Na}^+$  handling is altered in tumours, explore our current understanding of the mechanisms that may underlie these alterations and consider the potential consequences for cancer progression. Dysregulated  $\text{Na}^+$  balance in tumours may open opportunities for new imaging biomarkers and re-purposing of drugs for treatment.

## Introduction

The concentration of several key ions, including protons ( $H^+$ ) (1), potassium ( $K^+$ ) (2), calcium ( $Ca^{2+}$ ) (3) and sodium ( $Na^+$ ) (4) is altered in tumours. This ionic imbalance contributes to several cancer hallmarks, including altered growth signalling, proliferation, angiogenesis, invasion and metastasis (5). Just as intracellular ion concentrations can alter cancer cell behaviour, the extracellular “ionic tumour microenvironment” can determine how cancer, stromal and infiltrating immune cells behave (6). Dysregulation of ion homeostasis within the tumour microenvironment could therefore also contribute to tumour progression. Thus, ion channels and transporters, including those permeant to  $Na^+$ , have potential as novel targets for therapeutic intervention.

Control of  $Na^+$  is critical for normal cellular function and homeostatic dysregulation is a key feature of disease states such as acute inflammation (7) and ischaemia (8).  $Na^+$  handling is also altered in cancer:  $Na^+$  is raised in malignant tumours compared to corresponding healthy tissues (9). Tumorigenesis is accompanied by alterations to metabolism, pH regulation, vascularity and cell density that affect the distribution of  $Na^+$  within cancer cells and the extracellular tumour microenvironment. Here, we review the evidence showing that  $Na^+$  handling is altered in tumours, explore the mechanisms that may underlie these alterations and consider the potential consequences for cancer progression. We also highlight potential clinical applications for  $Na^+$  as a diagnostic biomarker and for targeting dysregulated  $Na^+$  within the ionic microenvironment of tumours alongside existing cancer therapeutics.

The extracellular  $Na^+$  concentration ( $[Na^+]_e$ ) is typically an order of magnitude higher (145 mM) than intracellular  $[Na^+]$  ( $[Na^+]_i$ ; 12 mM) (10). Therefore, an increase in total tumour tissue  $[Na^+]$  could be caused by an increase in the volume of extracellular fluid relative to the volume of intracellular fluid (extracellular volume fraction; Figure 1). The generation of

permeable vasculature in tumours by the angiogenic vascular endothelial growth factor (VEGF) allows leakage of plasma proteins, including glycosaminoglycans and collagen, into the interstitial space (11, 12). Protein leakage into the interstitium will also increase the colloid osmotic pressure, which contributes to the raised interstitial fluid pressure seen in solid tumours (13), potentially expanding the interstitial fluid volume (14, 15). Cell death following successful chemotherapeutic intervention would also be expected to increase the extracellular volume fraction. This would have implications for other ions. For example,  $K^+$  released into the extracellular space following tumour cell death results in an elevated extracellular  $[K^+]$  that suppresses the anti-tumour activity of tumour infiltrating lymphocytes (16). Nevertheless, the increase in interstitial fluid volume may underlie the raised total tumour  $[Na^+]$ . Although it has historically been difficult to measure accurately, there is some evidence that the interstitial fluid compartment is enlarged in some tumour types, including those which induce oedema, such as malignant gliomas and meningiomas (17-19).

Raised total tumour tissue  $[Na^+]$  may also be caused by increased  $[Na^+]_i$ , increased  $[Na^+]_e$ , or a combination of both. Quantitative measurement of tumour  $[Na^+]_e$  is lacking, however, there is evidence that  $[Na^+]_i$  is raised in tumours. Early studies using x-ray dispersion microanalysis and flame photometry indicated that the  $[Na^+]_i$  of cancer cells from various tumour types was more than double that of cells from adjacent healthy tissues (20, 21). Additional approaches, including measurement of  $^{22}Na$  radioisotope assimilation rate by atomic absorbance spectrophotometry, live cell imaging using the fluorescent  $Na^+$  reporter SBFI-AM, and  $^{23}Na$  magnetic resonance imaging ( $^{23}Na$  MRI; Box 1) have broadly confirmed these observations in cultured cells and cancer patients (22-27).

### **Cellular mechanisms underlying sodium handling in tumours**

Numerous plasma membrane channels and transporters facilitate  $Na^+$  flux down the electrochemical gradient from the extracellular space into the cytosol (Table 1; Figure 2).

Altered expression or activity of such mechanisms in cancer cells could account for the raised  $[\text{Na}^+]_i$  observed in tumours. The ubiquitous  $\text{Na}^+/\text{K}^+$  ATPase is almost exclusively responsible for the removal of  $\text{Na}^+$  from cells and thus maintaining the inward gradient for  $\text{Na}^+$ . As a result, this pump uses a significant proportion of the total ATP produced by non-excitable cells (28, 29). Altered cellular metabolic activity may thus lead to changes in  $\text{Na}^+/\text{K}^+$  ATPase activity. For example, when the usage of ATP for cellular proliferation is increased, the provision of ATP to the  $\text{Na}^+/\text{K}^+$  ATPase may be reduced and, as a consequence, changes in  $[\text{Na}^+]_i$  and  $[\text{Na}^+]_e$  would occur (30). Raised  $[\text{Na}^+]_i$  and possibly  $[\text{Na}^+]_e$  can both increase  $\text{Na}^+/\text{K}^+$  ATPase activity to maintain  $\text{Na}^+$  homeostasis (31-33). Since this pump is so energetically demanding, increasing  $\text{Na}^+$  influx would increase the cellular ATP consumption rate. Evidence suggests that the  $\text{Na}^+/\text{K}^+$  ATPase is predominantly fuelled by glycolysis in breast cancer cells since this delivers ATP quickly to the site where it is being used (34, 35). However, hypoxia has been shown to inhibit  $\text{Na}^+/\text{K}^+$  ATPase activity, suggesting that mitochondrial ATP supply is also needed (36, 37).

The inward electrochemical  $\text{Na}^+$  gradient set up by the  $\text{Na}^+/\text{K}^+$  ATPase powers the activity of a number of different  $\text{Na}^+$ -dependent transport mechanisms. The  $\text{Na}^+/\text{H}^+$  exchanger (NHE) family is one such mechanism, which uses the inward  $\text{Na}^+$  gradient to move  $\text{H}^+$  into the extracellular space, thus playing a central role in pH homeostasis (38). The ubiquitously expressed NHE1 is activated by receptor tyrosine kinase signalling, in particular via the Ras-extracellular signal-regulated kinase (ERK) pathway, and by osmotic stress, hormones and growth factors (39). NHE1 is also allosterically activated by an increase in intracellular  $[\text{H}^+]$ , as may be found in glycolytic tumour cells (40, 41). Another major pH regulation mechanism coupled to inward  $\text{Na}^+$  transport is the electroneutral  $\text{Na}^+/\text{HCO}_3^-$  cotransporter (NBCn1), which is upregulated in hypoxic tumours (42, 43). The imported  $\text{HCO}_3^-$  neutralises  $\text{H}^+$  generated from high metabolic activity by forming  $\text{H}_2\text{O}$  and  $\text{CO}_2$ . The  $\text{Na}^+$  gradient also powers the import of amino acids into cells via  $\text{Na}^+$ -dependent amino acid transporters, many of which are overexpressed in cancers (44).  $\text{Na}^+$ -dependent glucose transporters

(SGLTs), normally responsible for active glucose uptake in the kidney, are also functionally expressed in many cancers (45, 46). In addition, the  $\text{Na}^+\text{-K}^+\text{-Cl}^-$  cotransporter (NKCC), a key regulator of osmotic balance and cell volume which facilitates the transport of  $\text{Na}^+$ ,  $\text{K}^+$  and  $2\text{Cl}^-$  into the cell, is also upregulated in numerous cancers (47, 48). The combined activity of such nutrient and electrolyte transport mechanisms is not only dependent on  $\text{Na}^+$  homeostasis within the tumour microenvironment, but is also predicted to raise  $[\text{Na}^+]_i$ , thus potentially contributing to the elevated  $\text{Na}^+$  signal observed in tumours.

$\text{Na}^+$  channels expressed on tumour cells also enable  $\text{Na}^+$  influx and elevation of  $[\text{Na}^+]_i$ . Voltage-gated  $\text{Na}^+$  channels (VGSCs), classically expressed in electrically excitable cells where they initiate action potentials via  $\text{Na}^+$  influx, are also expressed in many tumour cell types where they promote cancer cell invasion and metastasis (49, 50). Although the voltage-dependent opening of these channels is transient, they also conduct a 'persistent' inward  $\text{Na}^+$  current under resting conditions, thus providing a route for  $\text{Na}^+$  to enter the cytosol in non-excitable tumour cells (51-55). The amiloride-sensitive epithelial  $\text{Na}^+$  channel (ENaC) and the related acid-sensing ion channels (ASICs) are also  $\text{Na}^+$ -selective ion channels which permit voltage-independent inward  $\text{Na}^+$  current. ENaC and ASICs have been linked to proliferation, migration, invasion and metastasis in various cancers (56, 57). Flow of  $\text{Na}^+$  through ENaC and ASICs is regulated by extracellular  $\text{H}^+$  (58, 59). Thus, both channels may contribute to elevation of  $[\text{Na}^+]_i$  in acidic tumours. In addition, N-methyl-D-aspartate (NMDA) receptors may also permit elevated  $[\text{Na}^+]_i$  in tumours. These ligand-gated, nonselective cation channels are typically expressed in the central nervous system (CNS) and activated by glutamate. NMDA receptors are expressed in numerous tumour types, including non-neuronal tumours such as pancreatic, breast and ovarian cancers, where they regulate invasion and correlate with poor prognosis (60-62). Proteins forming the G protein-coupled receptor-activated  $\text{Na}^+$  leak channel (NALCN) have been suggested as potential cancer susceptibility loci (63), and although evidence for its involvement in cancer is limited, NALCN may provide an additional route for  $\text{Na}^+$  influx, thus elevating  $[\text{Na}^+]_i$ . Finally, the two-

pore channel (TPC) family of lysosomal and endosomal cation channels can increase cytosolic  $\text{Ca}^{2+}$  and  $\text{Na}^+$  and have been shown to promote lung cancer cell migration (64) and epithelial-mesenchymal transition of breast cancer cells (65).

### **Pathophysiological consequences of altered tumour $\text{Na}^+$**

Dysregulated  $\text{Na}^+$  handling in tumours can lead to significant physiological changes at the cellular level, such as altered electrical potential difference across the plasma membrane (membrane potential;  $V_m$ ), pH, or metabolic activity. These physiological alterations can induce myriad effects on key tumour hallmarks from proliferative ability to invasion into healthy tissue and immune evasion (Figure 3).

#### ***Membrane potential depolarisation***

Influx of  $\text{Na}^+$  into non-excitabile cells depolarises the  $V_m$  (around 5-10 mV) (22, 66-68). In general, cancer cells exhibit a more depolarised  $V_m$  than their normal counterparts (around -5 to -50 mV vs. -50 to -95 mV), which may correlate with their increased proliferative capacity (69). This phenomenon may be due to the changes in  $V_m$  that accompany different stages of the cell cycle (70). Indeed, a relatively negative  $V_m$  ( $V_m$  hyperpolarisation) can prevent DNA synthesis and mitosis (71). Furthermore, stem cell differentiation can only occur if the  $V_m$  is hyperpolarised (72).  $V_m$  depolarisation leads to reorganisation of charged phospholipids in the inner leaflet of the plasma membrane, which in turn enhances nanoclustering and activation of K-Ras promoting mitogenic signalling (73).  $V_m$  depolarisation is also functionally instructive in regulating cytoskeletal reorganisation, morphogenesis, regeneration and tumorigenesis (68, 74-78). In effect, persistent  $\text{Na}^+$  entry via ENaCs and VGSCs may increase proliferation, maintain a poorly differentiated phenotype and increase migration via depolarisation of the  $V_m$ , all aiding tumour progression. However,  $V_m$  depolarisation in tumour cells is likely tightly regulated given that it can also



promote apoptosis and isotonic volume decrease (79, 80) and may thus present an interesting therapeutic target.

### ***Regulation of pH dynamics***

The extracellular microenvironment of solid tumours is commonly acidic (pH 6.5–7.2), whereas the intracellular pH of cancer cells is typically neutral or slightly alkaline (81, 82). The acidic tumour microenvironment is a critical contributory factor to many cancer hallmarks such as invasion, altered metabolism, drug resistance and immune evasion (83). The  $\text{Na}^+$  gradient across the plasma membrane impacts on pH regulation mechanisms. For example, an altered inward  $\text{Na}^+$  gradient will influence the pH-regulating capacity of cancer cells by regulating influx of  $\text{HCO}_3^-$  via NBCn1 and efflux of  $\text{H}^+$  via NHE1. NBCn1 is the predominant means of  $\text{H}^+$  extrusion from tumour cells when  $\text{pH}_i$  is  $> 6.6$ , whereas NHE1 is important under more acidic conditions, such as those observed in highly glycolytic cancer cells in a hypoxic tumour (42, 84, 85). Since both transport mechanisms are present in cancer cells (42, 86), they may work in tandem to facilitate  $\text{Na}^+$ -dependent tumour progression. Increased NHE1 activity leads to intracellular alkalinisation (87) and this may be a critical early event in oncogene-induced malignant transformation (88). Maintenance of a high  $\text{pH}_i$  by NHE1 activity is permissive for upregulation of both glycolytic activity and protein synthesis required for rapid cell growth and division (1, 89, 90). On the other hand, extracellular acidification promotes invasion and suppresses the immune response (91, 92). Thus, inhibition of the inward  $\text{Na}^+$  gradient may provide an effective intervention to manipulate tumour pH for therapeutic benefit.

The acidic pH of tumours may also reciprocally regulate  $\text{Na}^+$  conductance. For example, ENaC and ASIC channels are regulated by  $\text{pH}_e$  (58, 59) and the persistent inward  $\text{Na}^+$  current carried by VGSCs is increased under hypoxia or extracellular acidification (93, 94). Similarly, the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX) is also regulated by pH, with an acidic  $\text{pH}_i$  inhibiting forward ( $\text{Ca}^{2+}$  efflux/ $\text{Na}^+$  influx) mode action (95). Moreover, given that the sensitivity of NCX

to  $H^+$  requires intracellular  $Na^+$  (96), it is tempting to speculate that the altered pH and  $Na^+$  levels in tumours work in tandem to perturb  $Ca^{2+}$  signalling and homeostasis.

### ***Regulation of metabolic activity***

Altered tumour  $[Na^+]_i$  leads to changes in glucose metabolism that facilitate cancer progression. In a phenomenon first reported by Warburg in the 1920s (97), cancer cells exhibit upregulated glycolysis with conversion of glucose to lactic acid despite the presence of abundant oxygen ('aerobic glycolysis'). This shift in metabolism towards a more glycolytic phenotype confers numerous survival advantages for cancer cells, including survival within a hypoxic tumour core, and is associated with rapid cell proliferation, acidification of the tumour microenvironment, metastasis and poor patient outcome (98). In addition to directly regulating cancer cell metabolism via the hypoxia sensor HIF-1 $\alpha$ , tumour hypoxia may indirectly contribute to a highly glycolytic phenotype via elevation of tumour  $[Na^+]_i$ . Elevations in both tissue and intracellular  $[Na^+]_i$  are observed in ischaemic tissue (99), and hypoxia is known to increase the persistent inward  $Na^+$  current through VGSCs (100, 101). This increase in the persistent  $Na^+$  current would be expected to elevate  $[Na^+]_i$  in cancer cells expressing these channels. Moreover, in hypoxic tumours, upregulation of glycolysis and increased extrusion of  $H^+$  by NHE would increase  $[Na^+]_i$  (102).

*In vitro* studies indicate that elevations in  $[Na^+]_e$  can drive a highly glycolytic phenotype via the induction of various signalling pathways. For example, early studies revealed that glycolytic lactic acid production of HeLa cells increased as  $[Na^+]_e$  increased (103). Elevated  $[Na^+]_e$  upregulates the key cancer-associated glycolytic enzymes pyruvate kinase M2, lactate dehydrogenase A and hexokinase II, leading to increased glucose consumption and lactate production (104). Elevated  $[Na^+]_i$  may influence cancer cell metabolism due to increased energy demands from  $Na^+$  homeostasis mechanisms. Indeed,  $Na^+/K^+$  ATPase activity can also regulate the expression of glycolytic enzymes, and G-protein GPR35 mutations, which increase  $Na^+/K^+$  ATPase activity, increase the glycolytic rate (105). Conversely, inhibition of

the Na<sup>+</sup>/K<sup>+</sup> ATPase reduces expression of the hypoxia sensor HIF-1α, preventing it from upregulating glycolysis via increased expression of the glucose transporter GLUT-1 and hexokinase (106).

Altered [Na<sup>+</sup>]<sub>i</sub> may also directly affect mitochondrial metabolism by facilitating Ca<sup>2+</sup> transport between the mitochondria and the cytosol. The mitochondrial Na<sup>+</sup>/Ca<sup>2+</sup> (lithium) exchanger (NCLX) regulates mitochondrial Ca<sup>2+</sup> content by extruding Ca<sup>2+</sup> into the cytosol in exchange for Na<sup>+</sup> or Li<sup>+</sup> (107), and is regulated by the cytoplasmic [Na<sup>+</sup>]. Thus, NCLX uses Na<sup>+</sup> transport to fine-tune the mitochondrial [Ca<sup>2+</sup>], thereby regulating mitochondrial metabolism, redox homeostasis and ATP production (108, 109). Inhibition of NCLX induces apoptosis in prostate cancer cells (110), suggesting that an elevated [Na<sup>+</sup>]<sub>i</sub> in cancer cells might promote apoptosis resistance via NCLX. Taken together, these data suggest that elevated tumour [Na<sup>+</sup>] and Na<sup>+</sup>/K<sup>+</sup> ATPase activity contribute to a highly glycolytic cancer cell phenotype, which would be expected to promote proliferation, tumour acidification and resistance to apoptosis. However, the underlying mechanisms linking tumour [Na<sup>+</sup>] to cancer metabolism remain poorly characterised and require further research.

### ***Nutrient transport***

Amino acids regulate cancer cell signalling and metabolism (111), raising the possibility that altered amino acid uptake through Na<sup>+</sup>-dependent systems might influence cancer progression following changes to the transmembrane Na<sup>+</sup> gradient. For example, the Na<sup>+</sup>-dependent SGLT glucose transporters facilitate glucose uptake into cancer cells, and specific blockade of SGLT2 reduces mitochondrial ATP production and cellular proliferation and increases tumour necrosis (45, 112). Moreover, the Na<sup>+</sup>-dependent amino acid transporter SLC1A5, which is highly expressed in cancers and is driven by myc expression, imports glutamine (among other amino acids), and activates mammalian target of rapamycin complex 1 (mTORC1) to facilitate proliferation (113, 114). Many cancers, including triple-

negative breast cancer, have a “glutamine addiction” since this amino acid is a key carbon source for fatty acid production and mitochondrial ATP production (115, 116). Changes to the  $\text{Na}^+$  gradient may therefore regulate nutrient uptake in cancer cells and these observations raise the interesting possibility that pharmacologically reducing the inward  $\text{Na}^+$  gradient may impair the ability of cancer cells to import nutrients.

### ***Cell volume regulation***

$\text{Na}^+$  salts are the main contributors to the osmolarity of extracellular fluid, and the osmolarity of intracellular and extracellular fluids must be balanced to prevent cell shrinkage or swelling. The  $\text{Na}^+/\text{K}^+/\text{Cl}^-$  cotransporter NKCC1 activity is driven by the inward  $\text{Na}^+$  gradient and acts to regulate cell volume by facilitating the accumulation of intracellular  $\text{Cl}^-$  (117). Solid tumours exhibit a high interstitial colloid osmotic pressure (COP) and hydrostatic pressure in the interstitial fluid (13) which would be expected to hinder cell expansion. In vitro evidence shows that breast cancer cells in spheroids under compression actively extrude  $\text{Na}^+$  through NHE1 to reduce intracellular tonicity, leading to osmosis into the cell to resist compressive forces (118). In this circumstance, NHE1 functions in the reverse mode, importing  $\text{H}^+$  leading to intracellular acidification. Thus, by regulating  $\text{Na}^+$  as well as  $\text{H}^+$ , NHE1 activity must balance the cell's pH and volume regulation needs. In hypotonic conditions, cancer cells regulate  $[\text{Na}^+]_i$  to protect against volume increase (119). Therefore, tight regulation of  $\text{Na}^+$  transport is critical for maintaining cell volume in response to changes in COP and hydrostatic pressure in the tumour microenvironment.

### **Effects of $\text{Na}^+$ on tumour progression**

As a result of its impact on physiological behaviour of cancer and stromal cells, substantial experimental evidence supports the role of raised  $[\text{Na}^+]$  in promoting key aspects of tumour progression, including proliferation, migration, invasion and inflammation (22, 120-122) (Figure 4).

### ***Proliferation***

High osmolarity in the tumour microenvironment promotes proliferation via modulation of  $[Na^+]_i$ . Because the tumour interstitial fluid COP is higher than in healthy tissues (123, 124), and this contributes to a higher osmolarity, inward  $Na^+$  current is enhanced in cancer cells. For example, high osmolarity increases inward  $Na^+$  current through ENaC, which elevates  $[Na^+]_i$ , promotes tumour cell proliferation and inhibits apoptosis (121, 125, 126). This hyperosmolarity-induced inward current may promote proliferation by triggering brx-dependent activation of the small GTPase Rac1 thus stimulating the mitogen-activated protein kinase (MAPK)/ERK1/2 cascade (127, 128). High  $[Na^+]_e$  has also been shown to increase phosphorylation of the salt-inducible serine/threonine kinase SIK3 in breast cancer cells, which promotes proliferation via release from G1/S-phase arrest (129). Moreover, NKCC1 expression, cell shrinkage and  $Na^+$ -dependent  $Cl^-$  accumulation have been established as important regulators of the cell cycle and proliferation in cancer cells (48, 117, 130). On the other hand, moderate hypertonicity has been shown to lead to dormancy (131), suggesting that the level of osmotic pressure within tumours, and the cellular response to this, may be critical for determining fate. High  $[Na^+]_e$  has also been shown to induce DNA breaks and temporary cell cycle arrest (132). However, unlike most cases of DNA damage-induced cell cycle arrest, these DNA breaks are not repaired during this period, and the DNA damage persists when cells adapt to high  $[NaCl]_e$  and start to proliferate, with implications for oncogenesis (133-135).

### ***Migration***

Several  $Na^+$  transport systems have been shown to control tumour cell migration. For example, NHE1 has been shown to work in concert with aquaporins to allow cancer cells to move through confined spaces by taking in water and ions at the leading edge and expelling water from the trailing edge. This is achieved partly by concentrating NHE1 and aquaporin AQP5 at the leading edge of cells (136). Similarly, ENaC expression is increased at the

leading edge of migrating choriocarcinoma cells, promoting their motility (137). In addition, a glioma-specific  $\text{Na}^+$  channel made up of ENaC and ASIC subunits promotes migration and cell cycle progression (138). VGSC activity has also been shown to promote acquisition of a mesenchymal-like elongate morphology and thus increase migration of cells from a range of different types of cancer (139-144).

The  $\text{Na}^+$  gradient across the plasma membrane is tightly linked to  $\text{Ca}^{2+}$  transport by the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX), which is also upregulated in tumour cells (145, 146). NCX classically acts to extrude cytosolic  $\text{Ca}^{2+}$  following large increases in  $[\text{Ca}^{2+}]_i$ , thereby importing  $\text{Na}^+$  (147). Importantly, small changes to the  $\text{Na}^+$  gradient across the plasma membrane can alter the equilibrium potential for NCX and thereby lead to its operating in reverse ( $\text{Ca}^{2+}$  entry/ $\text{Na}^+$  exit) mode (148, 149). NCX reverse mode action has been implicated as a key mediator of transforming growth factor  $\beta$  (TGF- $\beta$ )-induced  $\text{Ca}^{2+}$ -dependent migration in hepatocellular and pancreatic cancer cells (150, 151). NCX may thus provide a mechanism linking elevations in  $[\text{Na}^+]_i$  to protumour  $\text{Ca}^{2+}$  signalling (149, 152). Interestingly, NCX inhibition has also been reported to decrease the intracellular accumulation of  $^{11}\text{C}$ -choline in cancer cells. This has important implications for positron emission tomography (PET) imaging, since alterations in tumour  $\text{Na}^+$  content might compromise NCX action and thus lead to poor contrast agent accumulation within tumours (153).

### ***Invasion***

A cancer hallmark commonly associated with aberrant  $\text{Na}^+$  homeostasis is the ability of cancer cells to invade into healthy tissues and migrate around the body to form metastases (49). Invasion requires proteolytic breakdown of the extracellular matrix by enzymes such as matrix metalloproteases and cathepsins. Cathepsins in particular are activated by low pH (154) so  $\text{Na}^+$ -dependent tumour acidification may thus facilitate invasion. NHE1 is expressed in the invadopodia of migrating breast cancer cells and colocalises with the invadopodial

marker cortactin (155). NHE1 activity in the invadopodia leads to local acidification of the extracellular compartment, providing the ideal pH conditions at the leading edge of an invading cell for digestion of the extracellular matrix (87, 120, 156-159).

VGSCs also promote the invasiveness and metastatic ability of a range of different cancer cell types (26, 141, 160, 161) and VGSC expression correlates with lymph node metastasis and a poor prognosis in breast cancer patients (54, 160). This appears to rely on the Na<sup>+</sup> conductance properties of VGSCs, since silencing VGSC expression or specific blockade results in decreased invasion *in vitro* (22, 161) and metastasis *in vivo* (162, 163). VGSCs facilitate an invasive phenotype by inducing transcriptional changes in genes contributing to Wnt, MAPK and Ca<sup>2+</sup> signalling (161, 164). VGSC activity may also increase cancer cell invasiveness through altering pH homeostasis. VGSC-dependent Na<sup>+</sup> influx in caveolae promotes H<sup>+</sup> extrusion by NHE1, thus acidifying the extracellular space and increasing cathepsin B protease activity (120, 142, 152). The authors proposed a putative allosteric interaction between VGSCs and NHE1 as the mechanism by which this interaction is mediated, since Na<sup>+</sup> influx would otherwise be expected to reduce the Na<sup>+</sup> gradient driving H<sup>+</sup> export by NHE1 (142).

ASICs are also upregulated in tumour cells where they promote pH-dependent migration and invasion (165). For example, ASIC1a increases [Ca<sup>2+</sup>]<sub>i</sub> and promotes migration as a result of pH<sub>e</sub> acidification (166) and ASIC2 mediates acidosis-induced invasion and metastasis (56). ASIC opening also results in activation of RhoA and induction of the epithelial-mesenchymal transition in pancreatic cancer cells (167). Co-expression of NMDA receptors and glutamate transporters (vGlut1–3) in glioma, pancreatic and ovarian cancer cells correlates with poor prognosis, suggesting an autocrine signalling mechanism that drives disease progression (60). Moreover, *in vitro* invasion and *in vivo* tumour burden are both decreased following treatment with a selective non-competitive NMDA receptor antagonist (60). Although these effects were attributed to Ca<sup>2+</sup> entry-induced activation of the Ca<sup>2+</sup>/calmodulin-dependent

protein kinase (CaMK) and mitogen-activated protein kinase kinase (MEK)-MAPK pathways, it remains to be determined whether elevated  $\text{Na}^+$  entry via NMDA receptors also contributes to their pro-invasive potential.

NKCC1 is also localised to the leading processes of migrating glioma cells, where it facilitates invasive behaviour by regulating focal adhesions,  $\text{Cl}^-$  accumulation and cell volume (47, 117, 168). The cell shrinkage that results from  $\text{Na}^+$ -linked  $\text{Cl}^-$  accumulation allows the invading cell to navigate narrow gaps in the peritumoural space. Interestingly, NKCC1 expression in hepatoma cells is upregulated in response to hyperosmolarity (169), suggesting that an elevation in tumour  $[\text{Na}^+]_e$  could promote an aggressive cell phenotype overexpressing NKCC1. These findings implicate NKCC1 as an important mediator of invasive potential and suggest that elevated  $\text{Na}^+$  may exacerbate metastatic behaviour by upregulating NKCC1 expression and  $\text{Na}^+$ -linked  $\text{Cl}^-$  accumulation.

### ***Tumour inflammation***

In sites of acute inflammation induced by inoculation with complete Freund adjuvant, BCG, or *Leishmania*,  $[\text{Na}^+]_e$  is increased (170, 171), leading to alterations in immune cell function (172, 173). The  $[\text{Na}^+]_e$  within inflamed solid tumours has not been studied, but it may be similarly altered. Chronic inflammation plays a critical role in cancer progression due to the abundance of cytokines and chemokines which stimulate proliferation and angiogenesis, and the release of reactive oxygen and nitrogen species from inflammatory cells which can cause DNA damage (174). Inflamed tissue has an increased extracellular osmolarity (171, 175, 176), and hyperosmolar conditions (e.g. high  $[\text{Na}^+]_e$ ) exacerbate inflammation (177, 178). Hyperosmotic stress is detected by many cell types including epithelial cells where it leads to activation of the nuclear factor of activated T-cells (NFAT-5) transcription factor. NFAT-5 is responsible for mediating integrin-induced breast cancer cell invasion (122). In macrophages, NFAT-5 activation in response to a hyperosmolar extracellular environment results in secretion of VEGF-C which stimulates angiogenesis (179). Hyperosmotic stress



upregulates production of inflammatory cytokines including interleukin (IL)-1 $\beta$ , IL-6, IL-8 and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) via the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) (171). High Na<sup>+</sup> in the microenvironment also provides a mitogenic stimulus to macrophages via activation of the p38 MAPK cascade (180). In addition, hyperosmolarity prolongs survival of macrophages by reducing production of the pro-apoptotic molecules p53 and bax (171). Tumour-associated macrophages (TAMs), which promote many aspects of cancer progression (181), may therefore be more prevalent in hyperosmotic tumour microenvironments. However, while evidence indicates that high [Na<sup>+</sup>]<sub>e</sub> stimulates an immune response in the skin via p38/MAPK and NFAT5 signalling and classical (M1) macrophage activation (170), studies have also shown that high [Na<sup>+</sup>]<sub>e</sub> induces peripheral macrophages to switch to an anti-inflammatory M2 (alternative activation) phenotype with poor phagocytic capacity, which would be expected to facilitate tumour progression rather than inhibit it (182). Based on these data, it appears that the regulation of the anti-tumour immune response by Na<sup>+</sup> is highly complex and may be tissue-specific (183).

Many pro-inflammatory effects of elevated [Na<sup>+</sup>]<sub>e</sub> are mediated by the cytokine IL-17. IL-17 is produced by CD4<sup>+</sup> T-helper 17 (Th17) cells in response to high [Na<sup>+</sup>]<sub>e</sub>, downstream of NFAT-5 activation (177). IL-17 signalling facilitates tumour development and progression (184-186). Thus, IL-17 induction by hyperosmotic stress links high [Na<sup>+</sup>]<sub>e</sub> to both tumour cell survival and metastasis. Indeed, elevated [Na<sup>+</sup>]<sub>e</sub> and IL-17 have been shown to induce expression of the promigratory VEGF-A (187). The synergistic proinflammatory effects of high [Na<sup>+</sup>]<sub>e</sub> and IL-17 can also be mediated by SIK3 to increase arginine metabolism, reactive nitrogen species production, CXCL-12 expression and matrix metalloproteinase (MMP9) activation (129). Interestingly, the high [Na<sup>+</sup>]<sub>e</sub>-induced component of the inflammatory response could be blocked by inhibiting Ca<sup>2+</sup> influx or by knockdown of the store-operated Ca<sup>2+</sup> entry (SOCE) regulatory molecules stromal interaction molecule

(STIM1) and Orai1, suggesting that these changes are governed by store-operated  $\text{Ca}^{2+}$  entry (188). These studies show that elevated  $[\text{Na}^+]_e$  can drive tumourigenicity via NFAT5, NF- $\kappa$ B, SIK3 activation and SOCE mechanisms.

## Diagnostic implications

The accumulating body of evidence implicating altered  $\text{Na}^+$  handling in regulating cancer cell behaviour, tumour metabolism, acidosis, growth, inflammation and invasion raises the intriguing possibility that the tumour  $[\text{Na}^+]$  may have value as a diagnostic or predictive biomarker in response to treatment. For example, measurement of  $[\text{Na}^+]_i$  may serve as a biomarker for both hypoxia-induced necrosis and cell death in response to successful chemotherapy treatments. Earlier detection of treatment response using techniques such as  $^{23}\text{Na}$  MRI (Box 1) may thus facilitate more timely selection of optimal therapies for individual patients, thereby improving clinical outcomes (189).  $^{23}\text{Na}$  MRI was first applied to supratentorial brain tumours in patients (190). Subsequently, this under-represented imaging methodology was used to show that the tissue  $[\text{Na}^+]$  is higher in malignant gliomas compared to normal brain tissue (4). A similar pattern was observed for breast (9, 191) and prostate tumours (25). This elevated tissue  $[\text{Na}^+]$  is present in both tumour tissue and oedema. Furthermore,  $[\text{Na}^+]$  is heterogeneous across the peritumoral region, suggesting that altered tissue  $[\text{Na}^+]$  may be demonstrating local physiological or biochemical changes within the tumour microenvironment (Figure 1) (9). Additionally, relaxation-weighted  $^{23}\text{Na}$ -MRI can differentiate between brain tumours of grades I-III and grade IV (192). However, while similar trends linking tumour grade to  $[\text{Na}^+]$  have been observed in breast and prostate cancers, any differences between tumour grades were below the threshold of statistical significance (25, 193). As such, future clinical studies with larger cohorts are needed to better determine the correlation between tumour  $[\text{Na}^+]$  and tumour type and grade.

A limitation with these observations using  $^{23}\text{Na}$  MRI is that they did not differentiate between  $\text{Na}^+$  located within the intracellular and extracellular compartments. To investigate this, Neto et.al. (2018) determined  $[\text{Na}^+]_i$  and  $[\text{Na}^+]_e$  in brain tumours (194). This revealed that although lesions have higher total  $[\text{Na}^+]$  than the normal appearing white matter, the extracellular volume fraction is also consistently elevated, whereas the apparent  $[\text{Na}^+]_i$  is lower than in white matter. This would imply that that increased extracellular volume may underlie the majority of the elevated tissue  $[\text{Na}^+]$ . However, relaxation-weighted  $^{23}\text{Na}$  MRI has shown that  $[\text{Na}^+]_i$  is elevated in glioblastomas and cerebral metastases (192) and is supported by in vitro data (22, 26). This observation of elevated  $[\text{Na}^+]_i$  has since been supported by additional studies in breast and prostate tumours using fluid suppression by inversion recovery and diffusion-weighted MRI approaches (25, 193, 195). Taken together, these studies suggest that both increases in extracellular volume fraction and  $[\text{Na}^+]_i$  can contribute to elevated total tissue  $[\text{Na}^+]$  in cancer, although the relative contributions of these two compartments may vary between tumour type and location.

$^{23}\text{Na}$  MRI may have the potential to complement standard of care radiological imaging approaches including positron emission tomography (PET). Elevated  $[\text{Na}^+]_i$  in brain tumours correlates with the proliferation marker MIB-1 (192), raising the possibility that  $^{23}\text{Na}$  MRI may complement  $^{18}\text{F}$ -fluorothymidine positron emission tomography ( $^{18}\text{F}$ FLT-PET) for proliferation assessment in CNS tumours (196). In addition,  $^{23}\text{Na}$  MRI measurement of total tumour  $[\text{Na}^+]$  has been shown to be superior to isocitrate dehydrogenase (IDH) mutation status in predicting progression-free survival, suggesting that tumour  $[\text{Na}^+]$  may be a promising tool for non-invasive outcome prediction (197). Furthermore,  $^{23}\text{Na}$  MRI may also have value for detecting changes in real-time during treatment as a potential early biomarker for therapy assessment (198). However, it must be noted that  $[\text{Na}^+]$  is likely to change following initiation of treatment as a result of physiological changes in the tumour and so the relationship between  $[\text{Na}^+]$  and therapy response may be complex. For example, in a preclinical mouse xenograft model of prostate cancer,  $[\text{Na}^+]_i$  increased within 24 h of

initiation of chemotherapy treatment (24), whereas in a rat glioma model,  $[\text{Na}^+]_i$  was significantly reduced 5 days after onset of chemotherapy (199). In broad agreement with the latter, total tumour  $[\text{Na}^+]$  is reduced in breast cancer patients responding to neoadjuvant chemotherapy (189, 200). However, the heterogeneity of  $[\text{Na}^+]$  within the tumour following therapy is likely to be critical. For example, chemotherapy-induced cellular necrosis would be expected to increase the extracellular volume fraction, likely underpinning the early increase in total tissue  $[\text{Na}^+]$  observed in preclinical tumour models following onset of therapy (201-203). Clearly, it is necessary to evaluate changes in both  $[\text{Na}^+]_i$  and  $[\text{Na}^+]_e$  in response to therapeutic intervention in order to evaluate predictive value of  $\text{Na}^+$  in the clinical setting. Whether MRI can do this remains to be explored across both preclinical and clinical theatres.

### **Therapeutic potential of manipulating $\text{Na}^+$ levels**

Directly or indirectly manipulating tumour  $\text{Na}^+$  levels, for example by using pharmacological tools to manipulate transporter activity, may present novel treatment options to complement existing therapeutics. Indeed, numerous studies are presently ongoing to evaluate targeting tumour  $[\text{Na}^+]$  (Table 2). Given that tumour acidification can induce drug resistance (1, 81, 204) and  $\text{Na}^+$  and pH are very closely linked, pharmacological modification of tumour  $[\text{Na}^+]$  may be a useful adjunct to other chemotherapeutics. For example, the ENaC/NHE1 blocker amiloride strongly synergises with doxorubicin to induce apoptosis and reduce glycolysis in osteosarcoma cells (205). On the other hand,  $\text{Na}^+/\text{K}^+$  ATPase activity is increased in hepatocarcinoma cells upon development of resistance to chemotherapeutics (206). Similarly, high  $[\text{Na}^+]_e$  increases expression of the multi-drug resistance protein P-glycoprotein in breast cancer cells in a  $\text{Ca}^{2+}$ -dependent manner (188). In addition, elevated  $[\text{Na}^+]$ -mediated acidification of the tumour microenvironment may help cancer cells to evade immune surveillance. Acidotic conditions correlate with low leukocyte counts (207) and decrease cytotoxicity of natural killer (NK) cells (208) and cytotoxic T-lymphocytes (209).

Thus, tumour  $[\text{Na}^+]_e$  may modulate response to existing chemotherapeutics and emerging immunotherapeutics via pH- and non pH-dependent mechanisms.

Extracellular  $[\text{Na}^+]$  in tumours would be expected to follow serum  $[\text{Na}^+]$  and indeed, hypertonic interstitial fluid accumulates in the skin of rats fed on a high salt diet (179). Since elevated tumour  $[\text{Na}^+]_e$  may promote tumour progression, serum  $[\text{Na}^+]$  is likely critical in cancer patients. Hyponatremia, which has a high mortality rate, is an uncommon side effect of some chemotherapy regimes (210). Significantly,  $\text{NaHCO}_3$  infusions, currently under test as a treatment to increase extracellular pH in cancer (Table 2), should be considered in the light of these findings. The risks associated with elevating  $[\text{Na}^+]_e$  (211) will need to be balanced against any advantages of reducing tumour acidity. Recently TRIS-base has been identified as a well-tolerated and effective anti-metastatic oral pH buffer in mice which does not require a counter-ion and would therefore not be expected raise  $[\text{Na}^+]_e$  (212).

Several studies have examined whether normalising tumour  $[\text{Na}^+]$  might be a useful treatment strategy. The use of VGSC inhibitors to prevent cancer growth and metastasis has been investigated in several preclinical studies (162, 213-215), and is currently the subject of several ongoing clinical trials (Table 2). In support of this, in retrospective observational studies, VGSC-inhibiting tricyclic antidepressants and antiepileptic medications have been shown to associate with reduced incidence of several common cancers including lung and colorectal cancer and glioma (216, 217). On the other hand, antiepileptic medications associate with increased mortality in breast, bowel and prostate cancer patients, although this may be a result of confounding by indication (218, 219). Significant improvements in cancer outcomes have been associated with local anaesthetic drugs such as lidocaine (220) and the anti-epileptic drug valproate in combination with doxorubicin (221) or a topoisomerase inhibitor (222); however the benefits may be attributed to other mechanisms in addition to VGSC inhibition. For example, regional anaesthesia reduces the need for general anaesthetic drugs and opioids, which have various deleterious effects on immunity

(223). The local anaesthetic lidocaine stimulates natural killer cell cytolytic activity (224) and several local anaesthetics inhibit src activity independently of VGSC blockade (225). As well as inhibiting VGSCs, valproic acid acts as a histone deacetylase inhibitor, and this action is commonly considered responsible for its anti-cancer properties (226). VGSCs are also inhibited by omega 3 fatty acids, which may explain some of the beneficial effects shown by these molecules in the diet of cancer patients (227, 228). A low  $\text{Na}^+$  diet, which reduces the risk of hypertension, has been hypothesised to be beneficial in preventing cancer (229). While speculative, this is supported by data from a meta-analysis of prospective studies examining habitual salt intake (230). However, a low  $\text{Na}^+$  diet may be difficult to achieve as a therapeutic intervention in the clinic as factors such as patient nutrition and blood pressure are already very difficult to control in patients with advanced disease. Furthermore, a complicating factor when considering VGSC inhibition as a therapeutic strategy is that these channels may also play a role in regulating immune cell function (231).

The reverse approach has also been considered: given that apoptosis is initiated by a large influx of  $\text{Na}^+$  into cells (232), attempts have been made to replicate this mechanism to kill cancer cells. Viral vector delivery of a constitutively open ASIC channel into culture glioma cells caused  $\text{Na}^+$  entry and cell death (233). However, viral delivery of  $\text{Na}^+$  channels would need to be precisely targeted to neoplastic cells in order for this method to be of use. In a different study, targeted osmotic lysis of VGSC-expressing tumours in mice was demonstrated by systemic administration of the  $\text{Na}^+/\text{K}^+$  ATPase-inhibiting cardiac glycoside ouabain in conjunction with electrical pulses to open VGSCs, leading to cytotoxic influx of  $\text{Na}^+$  into tumour cells (234). Blocking the  $\text{Na}^+$  efflux activity of  $\text{Na}^+/\text{K}^+$  ATPase with cardiac glycosides has the added advantage of decreasing inflammation and increasing specific anti-tumour immunity (235). A retrospective study showed improved survival in cancer patients that were being prescribed cardiac glycosides despite these patients having cardiac conditions (236). This has led to the development of several clinical trials examining the effect of adding the cardiac glycosides to various chemotherapy protocols (Table 2).

## Conclusion

$\text{Na}^+$  homeostasis is disrupted in cancer, leading to accumulation of  $\text{Na}^+$  in solid tumours. At the cellular level,  $\text{Na}^+$  transport is linked to pH and  $\text{Ca}^{2+}$  regulation and it alters plasma membrane potential, metabolism and proliferation. At the tissue level, high  $[\text{Na}^+]_i$  aids proliferation, migration and invasion of cancer cells and high  $[\text{Na}^+]_e$  induces an inflammatory microenvironment which promotes tumour progression. Systemic changes in  $[\text{Na}^+]$  affect blood pressure and immune function, together with secretion of pro-angiogenic mediators. Given that  $\text{Na}^+$  is the predominant extracellular cation and its distribution can be affected by diet and many drugs in common use, it is imperative that we further improve understanding of how  $\text{Na}^+$  regulation affects cancer progression. There is plenty of evidence that by doing so, we will uncover new modes of cancer detection and monitoring, e.g. through use of  $^{23}\text{Na}$ -MRI, and may also improve cancer treatment via pharmacological and dietary modulation of  $\text{Na}^+$  homeostasis.

## Acknowledgements

This work was supported by Cancer Research UK (A25922) and Breast Cancer Now (2015NovPhD572).

## Conflicts of interest statement

JDK reports grants from GSK, EU2020, and Cambridge Biomedical Research Centre, outside the submitted work. FAG reports grants from GSK and non-financial support from GE Healthcare. FDG reports grants from GE healthcare, personal fees from Google DeepMind, and non-financial support from Bracco, outside the submitted work. WJB reports grants from Cancer Research UK and Breast Cancer Now.

## References

1. Webb BA, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. *Nat Rev Cancer*. 2011;11(9):671-7.
2. Pardo LA, Stuhmer W. The roles of K(+) channels in cancer. *Nat Rev Cancer*. 2014;14(1):39-48.
3. Monteith GR, Prevarskaya N, Roberts-Thomson SJ. The calcium–cancer signalling nexus. *Nat Rev Cancer*. 2017;17(6):367-80.
4. Ouwerkerk R, Bleich KB, Gillen JS, Pomper MG, Bottomley PA. Tissue sodium concentration in human brain tumors as measured with <sup>23</sup>Na MR imaging. *Radiology*. 2003;227(2):529.
5. Prevarskaya N, Skryma R, Shuba Y. Ion channels and the hallmarks of cancer. *Trends Mol Med*. 2010;16(3):107-21.
6. Eil R, Vodnala SK, Clever D, Klebanoff CA, Sukumar M, Pan JH, et al. Ionic immune suppression within the tumour microenvironment limits T cell effector function. *Nature*. 2016;537(7621):539-43.
7. Biller A, Pflugmann I, Badde S, Diem R, Wildemann B, Nagel AM, et al. Sodium MRI in Multiple Sclerosis is Compatible with Intracellular Sodium Accumulation and Inflammation-Induced Hyper-Cellularity of Acute Brain Lesions. *Sci Rep*. 2016;6:31269.
8. Murphy E, Eisner DA. Regulation of intracellular and mitochondrial sodium in health and disease. *Circ Res*. 2009;104(3):292-303.
9. Ouwerkerk R, Jacobs MA, Macura KJ, Wolff AC, Stearns V, Mezban SD, et al. Elevated tissue sodium concentration in malignant breast lesions detected with non-invasive <sup>23</sup>Na MRI. *Breast Cancer Res Treat*. 2007;106(2):151-60.
10. Hille B. Ion channels of excitable membranes. 3rd ed. Sunderland, Massachusetts: Sinauer Associates Inc. ; 2001.
11. Dvorak HF, Nagy JA, Feng D, Brown LF, Dvorak AM. Vascular Permeability Factor/Vascular Endothelial Growth Factor and the Significance of Microvascular Hyperpermeability in Angiogenesis. *Current Topics in Microbiology and Immunology* 1999. p. 97-132.
12. Wiig H, Swartz MA. Interstitial fluid and lymph formation and transport: physiological regulation and roles in inflammation and cancer. *Physiol Rev*. 2012;92(3):1005-60.
13. Heldin CH, Rubin K, Pietras K, Ostman A. High interstitial fluid pressure - an obstacle in cancer therapy. *Nat Rev Cancer*. 2004;4(10):806-13.
14. Leu AJ, Berk DA, Lymboussaki A, Alitalo K, Jain RK. Absence of functional lymphatics within a murine sarcoma: a molecular and functional evaluation. *Cancer Res*. 2000;60(16):4324-7.
15. Padera TP, Kadambi A, di Tomaso E, Carreira CM, Brown EB, Boucher Y, et al. Lymphatic metastasis in the absence of functional intratumor lymphatics. *Science*. 2002;296(5574):1883-6.
16. Vodnala SK, Eil R, Kishton RJ, Sukumar M, Yamamoto TN, Ha NH, et al. T cell stemness and dysfunction in tumors are triggered by a common mechanism. *Science*. 2019;363(6434):eaau0135.
17. Kim YR, Savellano MD, Savellano DH, Weissleder R, Bogdanov A, Jr. Measurement of tumor interstitial volume fraction: method and implication for drug delivery. *Magn Reson Med*. 2004;52(3):485-94.
18. Jain RK. Transport of molecules in the tumor interstitium: a review. *Cancer Res*. 1987;47(12):3039-51.
19. Papadopoulos MC, Saadoun S, Binder DK, Manley GT, Krishna S, Verkman AS. Molecular mechanisms of brain tumor edema. *Neuroscience*. 2004;129(4):1009-18.
20. Cameron IL, Smith NK, Pool TB, Sparks RL. Intracellular concentration of sodium and other elements as related to mitogenesis and oncogenesis in vivo. *Cancer Res*. 1980;40(5):1493-500.



21. Hürter T, Bröcker W, Bosma HJ. Investigations on vasogenic and cytotoxic brain edema, comparing results from X-ray microanalysis and flame photometry. *Microsc Acta*. 1982;85(3):285-93.
22. Campbell TM, Main MJ, Fitzgerald EM. Functional expression of the voltage-gated Na(+)-channel Nav1.7 is necessary for EGF-mediated invasion in human non-small cell lung cancer cells. *J Cell Sci*. 2013;126(Pt 21):4939-49.
23. Wiley JS, Dubyak GR. Extracellular adenosine triphosphate increases cation permeability of chronic lymphocytic leukemic lymphocytes. *Blood*. 1989;73(5):1316-23.
24. Kline RP, Wu EX, Petrylak DP, Szabolcs M, Alderson PO, Weisfeldt ML, et al. Rapid in vivo monitoring of chemotherapeutic response using weighted sodium magnetic resonance imaging. *Clin Cancer Res*. 2000;6(6):2146-56.
25. Barrett T, Riemer F, McLean MA, Kaggie J, Robb F, Tropp JS, et al. Quantification of Total and Intracellular Sodium Concentration in Primary Prostate Cancer and Adjacent Normal Prostate Tissue With Magnetic Resonance Imaging. *Invest Radiol*. 2018;53(8):450-6.
26. Roger S, Rollin J, Barascu A, Besson P, Raynal PI, Iochmann S, et al. Voltage-gated sodium channels potentiate the invasive capacities of human non-small-cell lung cancer cell lines. *Int J Biochem Cell Biol*. 2007;39(4):774-86.
27. Yang M, James AD, Suman R, Kasprovicz R, Nelson M, O'Toole PJ, et al. Voltage-dependent activation of Rac1 by Nav1.5 channels promotes cell migration. *bioRxiv*. 2019:597088.
28. Nobes CD, Lakin-Thomas PL, Brand MD. The contribution of ATP turnover by the Na<sup>+</sup>/K<sup>+</sup>-ATPase to the rate of respiration of hepatocytes. Effects of thyroid status and fatty acids. *Biochim Biophys Acta*. 1989;976(2-3):241-5.
29. Buttgerit F, Brand MD. A hierarchy of ATP-consuming processes in mammalian cells. *Biochem J*. 1995;312 ( Pt 1):163-7.
30. Madelin G, Regatte RR. Biomedical applications of sodium MRI in vivo. *J Magn Reson Imaging*. 2013;38(3):511-29.
31. Pellerin L, Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci U S A*. 1994;91(22):10625-9.
32. Chatton JY, Marquet P, Magistretti PJ. A quantitative analysis of L-glutamate-regulated Na<sup>+</sup> dynamics in mouse cortical astrocytes: implications for cellular bioenergetics. *Eur J Neurosci*. 2000;12(11):3843-53.
33. Garcia A, Fry N, Karimi K, Liu C, Apell HJ, Rasmussen H, et al. Extracellular Allosteric Na<sup>+</sup> Binding to the Na<sup>+</sup>,K<sup>+</sup>-ATPase in Cardiac Myocytes. *Biophys J*. 2013;105(12):2695-705.
34. Epstein T, Xu L, Gillies RJ, Gatenby RA. Separation of metabolic supply and demand: aerobic glycolysis as a normal physiological response to fluctuating energetic demands in the membrane. *Cancer Metab*. 2014;2:7.
35. James AD, Patel W, Butt Z, Adiamah M, Dakhel R, Latif A, et al. The Plasma Membrane Calcium Pump in Pancreatic Cancer Cells Exhibiting the Warburg Effect Relies on Glycolytic ATP. *J Biol Chem*. 2015;290(41):24760-71.
36. Heerlein K, Schulze A, Hotz L, Bartsch P, Mairbaur H. Hypoxia decreases cellular ATP demand and inhibits mitochondrial respiration of A549 cells. *Am J Respir Cell Mol Biol*. 2005;32(1):44-51.
37. Zhou G, Dada LA, Chandel NS, Iwai K, Lecuona E, Ciechanover A, et al. Hypoxia-mediated Na-K-ATPase degradation requires von Hippel Lindau protein. *The FASEB Journal*. 2008;22(5):1335-42.
38. Orlowski J, Grinstein S. Na<sup>+</sup>/H<sup>+</sup> exchangers of mammalian cells. *J Biol Chem*. 1997;272(36):22373-6.
39. Putney LK, Denker SP, Barber DL. The changing face of the Na<sup>+</sup>/H<sup>+</sup> exchanger, NHE1: structure, regulation, and cellular actions. *Annu Rev Pharmacol Toxicol*. 2002;42:527-52.

40. Tekpli X, Huc L, Lacroix J, Rissel M, Poet M, Noel J, et al. Regulation of Na<sup>+</sup>/H<sup>+</sup> exchanger 1 allosteric balance by its localization in cholesterol- and caveolin-rich membrane microdomains. *J Cell Physiol.* 2008;216(1):207-20.
41. Gore J, Besson P, Hoinard C, Bougnoux P. Na<sup>+</sup>-H<sup>+</sup> antiporter activity in relation to membrane fatty acid composition and cell proliferation. *Am J Physiol.* 1994;266(1 Pt 1):C110-20.
42. Boedtker E, Moreira JMA, Mele M, Vahl P, Wielenga VT, Christiansen PM, et al. Contribution of Na<sup>+</sup>,HCO<sub>3</sub><sup>-</sup>-cotransport to cellular pH control in human breast cancer: a role for the breast cancer susceptibility locus NBCn1 (SLC4A7). *Int J Cancer.* 2013;132(6):1288-99.
43. McIntyre A, Hulikova A, Ledaki I, Snell C, Singleton D, Steers G, et al. Disrupting Hypoxia-Induced Bicarbonate Transport Acidifies Tumor Cells and Suppresses Tumor Growth. *Cancer Res.* 2016;76(13):3744-55.
44. Nakanishi T, Tamai I. Solute carrier transporters as targets for drug delivery and pharmacological intervention for chemotherapy. *J Pharm Sci.* 2011;100(9):3731-50.
45. Scafoglio C, Hirayama BA, Kepe V, Liu J, Ghezzi C, Satyamurthy N, et al. Functional expression of sodium-glucose transporters in cancer. *Proc Natl Acad Sci U S A.* 2015;112(30):E4111-9.
46. Ishikawa N, Oguri T, Isobe T, Fujitaka K, Kohno N. SGLT gene expression in primary lung cancers and their metastatic lesions. *Jpn J Cancer Res.* 2001;92(8):874-9.
47. Garzon-Muvdi T, Schiapparelli P, ap Rhys C, Guerrero-Cazares H, Smith C, Kim DH, et al. Regulation of brain tumor dispersal by NKCC1 through a novel role in focal adhesion regulation. *PLoS Biol.* 2012;10(5):e1001320.
48. Shiozaki A, Nako Y, Ichikawa D, Konishi H, Komatsu S, Kubota T, et al. Role of the Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> cotransporter NKCC1 in cell cycle progression in human esophageal squamous cell carcinoma. *World J Gastroenterol.* 2014;20(22):6844-59.
49. Brackenbury WJ. Voltage-gated sodium channels and metastatic disease. *Channels (Austin).* 2012;6(5):352-61.
50. Roger S, Gillet L, Le Guennec JY, Besson P. Voltage-gated sodium channels and cancer: is excitability their primary role? *Front Pharmacol.* 2015;6.
51. Alzheimer C, Schwindt PC, Crill WE. Modal gating of Na<sup>+</sup> channels as a mechanism of persistent Na<sup>+</sup> current in pyramidal neurons from rat and cat sensorimotor cortex. *J Neurosci.* 1993;13(2):660-73.
52. Eijkelkamp N, Linley JE, Baker MD, Minett MS, Cregg R, Werdehausen R, et al. Neurological perspectives on voltage-gated sodium channels. *Brain.* 1352012. p. 2585-612.
53. Djamgoz MB, Onkal R. Persistent current blockers of voltage-gated sodium channels: a clinical opportunity for controlling metastatic disease. *Recent Pat Anticancer Drug Discov.* 2013;8(1):66-84.
54. Yang M, Kozminski DJ, Wold LA, Modak R, Calhoun JD, Isom LL, et al. Therapeutic potential for phenytoin: targeting Na(v)1.5 sodium channels to reduce migration and invasion in metastatic breast cancer. *Breast Cancer Res Treat.* 2012;134(2):603-15.
55. Roger S, Besson P, Le Guennec JY. Involvement of a novel fast inward sodium current in the invasion capacity of a breast cancer cell line. *Biochim Biophys Acta.* 2003;1616(2):107-11.
56. Zhou Z, Song J, Li W, Liu X, Cao L, Wan L, et al. The acid-sensing ion channel, ASIC2, promotes invasion and metastasis of colorectal cancer under acidosis by activating the calcineurin/NFAT1 axis. *J Exp Clin Cancer Res.* 362017.
57. Xu S, Liu C, Ma Y, Ji HL, Li X. Potential Roles of Amiloride-Sensitive Sodium Channels in Cancer Development. *Biomed Res Int.* 2016;2016:2190216.
58. Boscardin E, Alijevic O, Hummler E, Frateschi S, Kellenberger S. The function and regulation of acid-sensing ion channels (ASICs) and the epithelial Na channel (ENaC): IUPHAR Review 19. *Br J Pharmacol.* 2016;173(18):2671-701.
59. Collier DM, Snyder PM. Extracellular protons regulate human ENaC by modulating Na<sup>+</sup> self-inhibition. *J Biol Chem.* 2009;284(2):792-8.

60. Li L, Hanahan D. Hijacking the neuronal NMDAR signaling circuit to promote tumor growth and invasion. *Cell*. 2013;153(1):86-100.
61. Li L, Zeng Q, Bhutkar A, Galvan JA, Karamitopoulou E, Noordermeer D, et al. GKAP Acts as a Genetic Modulator of NMDAR Signaling to Govern Invasive Tumor Growth. *Cancer Cell*. 2018;33(4):736-51.e5.
62. Stepulak A, Luksch H, Gebhardt C, Uckermann O, Marzahn J, Siffringer M, et al. Expression of glutamate receptor subunits in human cancers. *Histochem Cell Biol*. 2009;132(4):435-45.
63. Cochet-Bissuel M, Lory P, Monteil A. The sodium leak channel, NALCN, in health and disease. *Front Cell Neurosci*. 2014;8:132.
64. Nguyen ON, Grimm C, Schneider LS, Chao YK, Atzberger C, Bartel K, et al. Two-Pore Channel Function Is Crucial for the Migration of Invasive Cancer Cells. *Cancer Res*. 2017;77(6):1427-38.
65. Jahidin AH, Stewart TA, Thompson EW, Roberts-Thomson SJ, Monteith GR. Differential effects of two-pore channel protein 1 and 2 silencing in MDA-MB-468 breast cancer cells. *Biochem Biophys Res Commun*. 2016;477(4):731-6.
66. Stys PK, Sontheimer H, Ransom BR, Waxman SG. Noninactivating, tetrodotoxin-sensitive Na<sup>+</sup> conductance in rat optic nerve axons. *Proc Natl Acad Sci U S A*. 1993;90(15):6976-80.
67. Sontheimer H, Fernandez-Marques E, Ullrich N, Pappas CA, Waxman SG. Astrocyte Na<sup>+</sup> channels are required for maintenance of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity. *J Neurosci*. 1994;14(5 Pt 1):2464-75.
68. Chifflet S, Hernandez JA, Grasso S. A possible role for membrane depolarization in epithelial wound healing. *Am J Physiol Cell Physiol*. 2005;288(6):C1420-30.
69. Yang M, Brackenbury WJ. Membrane potential and cancer progression. *Front Physiol*. 2013;4:185.
70. Sachs HG, Stambrook PJ, Ebert JD. Changes in membrane potential during the cell cycle. *Exp Cell Res*. 1974;83(2):362-6.
71. Cone CD, Jr. Variation of the transmembrane potential level as a basic mechanism of mitosis control. *Oncology*. 1970;24(6):438-70.
72. Sundelacruz S, Levin M, Kaplan DL. Membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells. *PLoS One*. 2008;3(11):e3737.
73. Zhou Y, Wong CO, Cho KJ, van der Hoeven D, Liang H, Thakur DP, et al. Membrane potential modulates plasma membrane phospholipid dynamics and K-Ras signaling. *Science*. 2015;349(6250):873-6.
74. Nin V, Hernandez JA, Chifflet S. Hyperpolarization of the plasma membrane potential provokes reorganization of the actin cytoskeleton and increases the stability of adherens junctions in bovine corneal endothelial cells in culture. *Cell Motil Cytoskeleton*. 2009;66(12):1087-99.
75. Szaszi K, Sirokmany G, Di Ciano-Oliveira C, Rotstein OD, Kapus A. Depolarization induces Rho-Rho kinase-mediated myosin light chain phosphorylation in kidney tubular cells. *Am J Physiol Cell Physiol*. 2005;289(3):C673-85.
76. Beane WS, Morokuma J, Lemire JM, Levin M. Bioelectric signaling regulates head and organ size during planarian regeneration. *Development*. 2013;140(2):313-22.
77. Lobikin M, Chernet B, Lobo D, Levin M. Resting potential, oncogene-induced tumorigenesis, and metastasis: the bioelectric basis of cancer in vivo. *Phys Biol*. 2012;9(6):065002.
78. Chernet BT, Levin M. Transmembrane voltage potential of somatic cells controls oncogene-mediated tumorigenesis at long-range. *Oncotarget*. 2014;5(10):3287-306.
79. Bortner CD, Gomez-Angelats M, Cidlowski JA. Plasma membrane depolarization without repolarization is an early molecular event in anti-Fas-induced apoptosis. *J Biol Chem*. 2001;276(6):4304-14.
80. Lang F, Föller M, Lang K, Lang P, Ritter M, Vereninov A, et al. Cell volume regulatory ion channels in cell proliferation and cell death. *Methods Enzymol*. 2007;428:209-25.

81. Gerweck LE, Seetharaman K. Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. *Cancer Res.* 1996;56(6):1194-8.
82. Gillies RJ, Martinez-Zaguilan R, Martinez GM, Serrano R, Perona R. Tumorigenic 3T3 cells maintain an alkaline intracellular pH under physiological conditions. *Proc Natl Acad Sci U S A.* 1990;87(19):7414-8.
83. Corbet C, Feron O. Tumour acidosis: from the passenger to the driver's seat. *Nat Rev Cancer.* 2017;17(10):577-93.
84. Hulikova A, Harris AL, Vaughan-Jones RD, Swietach P. Regulation of intracellular pH in cancer cell lines under normoxia and hypoxia. *J Cell Physiol.* 2013;228(4):743-52.
85. Hulikova A, Vaughan-Jones RD, Swietach P. Dual role of CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> buffer in the regulation of intracellular pH of three-dimensional tumor growths. *J Biol Chem.* 2011;286(16):13815-26.
86. Lauritzen G, Stock C-M, Lemaire J, Lund SF, Jensen MF, Damsgaard B, et al. The Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1, but not the Na<sup>+</sup>, cotransporter NBCn1, regulates motility of MCF7 breast cancer cells expressing constitutively active ErbB2. *Cancer Lett.* 2012;317(2):172-83.
87. Cardone RA, Casavola V, Reshkin SJ. The role of disturbed pH dynamics and the Na<sup>+</sup>/H<sup>+</sup> exchanger in metastasis. *Nat Rev Cancer.* 2005;5(10):786-95.
88. Reshkin SJ, Bellizzi A, Caldeira S, Albarani V, Malanchi I, Poignee M, et al. Na<sup>+</sup>/H<sup>+</sup> exchanger-dependent intracellular alkalinization is an early event in malignant transformation and plays an essential role in the development of subsequent transformation-associated phenotypes. *Faseb j.* 2000;14(14):2185-97.
89. Reshkin SJ, Greco MR, Cardone RA. Role of pHi, and proton transporters in oncogene-driven neoplastic transformation. *Philos Trans R Soc Lond B Biol Sci.* 2014;369(1638):20130100.
90. Chambard JC, Pouyssegur J. Intracellular pH controls growth factor-induced ribosomal protein S6 phosphorylation and protein synthesis in the G<sub>0</sub>----G<sub>1</sub> transition of fibroblasts. *Exp Cell Res.* 1986;164(2):282-94.
91. Calcinotto A, Filipazzi P, Grioni M, Iero M, De Milito A, Ricupito A, et al. Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes. *Cancer Res.* 2012;72(11):2746-56.
92. Estrella V, Chen T, Lloyd M, Wojtkowiak J, Cornnell HH, Ibrahim-Hashim A, et al. Acidity generated by the tumor microenvironment drives local invasion. *Cancer Res.* 2013;73(5):1524-35.
93. Woodhull AM. Ionic blockage of sodium channels in nerve. *J Gen Physiol.* 1973;61(6):687-708.
94. Hammarstrom AK, Gage PW. Inhibition of oxidative metabolism increases persistent sodium current in rat CA1 hippocampal neurons. *J Physiol.* 1998;510 ( Pt 3):735-41.
95. Philipson KD, Bersohn MM, Nishimoto AY. Effects of pH on Na<sup>+</sup>-Ca<sup>2+</sup> exchange in canine cardiac sarcolemmal vesicles. *Circ Res.* 1982;50(2):287-93.
96. Doering AE, Lederer WJ. The mechanism by which cytoplasmic protons inhibit the sodium-calcium exchanger in guinea-pig heart cells. *J Physiol.* 1993;466:481-99.
97. Warburg O. On the Origin of Cancer Cells. *Science.* 1956;123(3191):309-14.
98. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nat Rev Cancer.* 2004;4(11):891-9.
99. Regan TJ, Broisman L, Haider B, Eaddy C, Oldewurtel HA. Dissociation of myocardial sodium and potassium alterations in mild versus severe ischemia. *Am J Physiol.* 1980;238(4):H575-80.
100. Ju YK, Saint DA, Gage PW. Hypoxia increases persistent sodium current in rat ventricular myocytes. *J Physiol.* 1996;497 ( Pt 2)(2):337-47.
101. Hammarstrom AK, Gage PW. Hypoxia and persistent sodium current. *Eur Biophys J.* 2002;31(5):323-30.
102. Stubbs M, Veech RL, Griffiths JR. Tumor metabolism: the lessons of magnetic resonance spectroscopy. *Adv Enzyme Regul.* 1995;35:101-15.

103. Stubblefield E, Mueller GC. Effects of Sodium Chloride Concentration on Growth, Biochemical Composition, and Metabolism of HeLa Cells. *Cancer Res.* 1960;20(11):1646-55.
104. Amara S, Zheng M, Tiriveedhi V. Oleanolic Acid Inhibits High Salt-Induced Exaggeration of Warburg-like Metabolism in Breast Cancer Cells. *Cell Biochem Biophys.* 2016;74(3):427-34.
105. Schneditz G, Elias JE, Pagano E, Zaeem Cader M, Saveljeva S, Long K, et al. GPR35 promotes glycolysis, proliferation, and oncogenic signaling by engaging with the sodium potassium pump. *Sci Signal.* 2019;12(562).
106. Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, et al. Digoxin and other cardiac glycosides inhibit HIF-1 synthesis and block tumor growth. *Proceedings of the National Academy of Sciences.* 2008;105(50):19579-86.
107. Palty R, Silverman WF, Hershfinkel M, Caporale T, Sensi SL, Parnis J, et al. NCLX is an essential component of mitochondrial Na<sup>+</sup>/Ca<sup>2+</sup> exchange. *Proc Natl Acad Sci U S A.* 2010;107(1):436-41.
108. Di Benedetto G, Di Benedetto G, Scalzotto E, Mongillo M, Pozzan T. Mitochondrial Ca<sup>2+</sup> Uptake Induces Cyclic AMP Generation in the Matrix and Modulates Organelle ATP Levels. *Cell Metab.* 2013;17(6):965-75.
109. De Marchi U, Santo-Domingo J, Castelbou C, Sekler I, Wiederkehr A, Demaurex N. NCLX protein, but not LETM1, mediates mitochondrial Ca<sup>2+</sup> extrusion, thereby limiting Ca<sup>2+</sup>-induced NAD(P)H production and modulating matrix redox state. *J Biol Chem.* 2014;289(29):20377-85.
110. Kaddour-Djebbar I, Lakshmikanthan V, Shirley RB, Ma Y, Lewis RW, Kumar MV. Therapeutic advantage of combining calcium channel blockers and TRAIL in prostate cancer. *Mol Cancer Ther.* 2006;5(8):1958-66.
111. Tsun ZY, Possemato R. Amino acid management in cancer. *Semin Cell Dev Biol.* 2015;43:22-32.
112. Villani LA, Smith BK, Marcinko K, Ford RJ, Broadfield LA, Green AE, et al. The diabetes medication Canagliflozin reduces cancer cell proliferation by inhibiting mitochondrial complex-I supported respiration. *Mol Metab.* 2016;5(10):1048-56.
113. Scalise M, Pochini L, Galluccio M, Console L, Indiveri C. Glutamine Transport and Mitochondrial Metabolism in Cancer Cell Growth. *Front Oncol.* 2017;7:306.
114. Bhutia YD, Babu E, Ramachandran S, Ganapathy V. Amino Acid transporters in cancer and their relevance to "glutamine addiction": novel targets for the design of a new class of anticancer drugs. *Cancer Res.* 2015;75(9):1782-8.
115. Cha YJ, Kim E-S, Koo JS. Amino Acid Transporters and Glutamine Metabolism in Breast Cancer. *Int J Mol Sci.* 2018;19(3).
116. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* 2009;324(5930):1029-33.
117. Habela CW, Ernest NJ, Swindall AF, Sontheimer H. Chloride accumulation drives volume dynamics underlying cell proliferation and migration. *J Neurophysiol.* 2009;101(2):750-7.
118. McGrail DJ, McAndrews KM, Brandenburg CP, Ravikumar N, Kieu QMN, Dawson MR. Osmotic Regulation Is Required for Cancer Cell Survival under Solid Stress. *Biophys J.* 2015;109(7):1334-7.
119. Rouzaire-Dubois B, Ouanounou G, O'Regan S, Dubois J-M. Sodium-dependent activity of aquaporin-1 in rat glioma cells: a new mechanism of cell volume regulation. *Pflügers Archiv - European Journal of Physiology.* 2008;457(5):1187-98.
120. Brisson L, Gillet L, Calaghan S, Besson P, Le Guennec JY, Roger S, et al. Na(V)1.5 enhances breast cancer cell invasiveness by increasing NHE1-dependent H(+) efflux in caveolae. *Oncogene.* 2011;30(17):2070-6.
121. Bondarava M, Li T, Endl E, Wehner F. alpha-ENaC is a functional element of the hypertonicity-induced cation channel in HepG2 cells and it mediates proliferation. *Pflugers Arch.* 2009;458(4):675-87.

122. Jauliac S, López-Rodríguez C, Shaw LM, Brown LF, Rao A, Toker A. The role of NFAT transcription factors in integrin-mediated carcinoma invasion. *Nat Cell Biol.* 2002;4(7):540-4.
123. Stohrer M, Boucher Y, Stangassinger M, Jain RK. Oncotic pressure in solid tumors is elevated. *Cancer Res.* 2000;60(15):4251-5.
124. Wiig H, Aukland K, Tenstad O. Isolation of interstitial fluid from rat mammary tumors by a centrifugation method. *Am J Physiol Heart Circ Physiol.* 2003;284(1):H416-24.
125. Sparks RL, Pool TB, Smith NK, Cameron IL. Effects of amiloride on tumor growth and intracellular element content of tumor cells in vivo. *Cancer Res.* 1983;43(1):73-7.
126. Vila-Carriles WH, Kovacs GG, Jovov B, Zhou Z-H, Pahwa AK, Colby G, et al. Surface expression of ASIC2 inhibits the amiloride-sensitive current and migration of glioma cells. *J Biol Chem.* 2006;281(28):19220-32.
127. Aramburu J, López-Rodríguez C. Brx shines a light on the route from hyperosmolarity to NFAT5. *Sci Signal.* 2009;2(65):e20.
128. Shen M-R, Chou C-Y, Hsu K-F, Clive Ellory J. Osmotic Shrinkage of Human Cervical Cancer Cells Induces an Extracellular Cl<sup>-</sup>-dependent Nonselective Cation Channel, Which Requires p38 MAPK. *J Biol Chem.* 2002;277(48):45776-84.
129. Amara S, Majors C, Roy B, Hill S, Rose KL, Myles EL, et al. Critical role of SIK3 in mediating high salt and IL-17 synergy leading to breast cancer cell proliferation. *PLoS One.* 2017;12(6):e0180097.
130. Hiraoka K, Miyazaki H, Niisato N, Iwasaki Y, Kawauchi A, Miki T, et al. Chloride ion modulates cell proliferation of human androgen-independent prostatic cancer cell. *Cell Physiol Biochem.* 2010;25(4-5):379-88.
131. Havard M, Dautry F, Tchénio T. A dormant state modulated by osmotic pressure controls clonogenicity of prostate cancer cells. *J Biol Chem.* 2011;286(51):44177-86.
132. Zhang Z, Ferraris JD, Irarrazabal CE, Dmitrieva NI, Park J-H, Burg MB. Ataxia telangiectasia-mutated, a DNA damage-inducible kinase, contributes to high NaCl-induced nuclear localization of transcription factor TonEBP/OREBP. *Am J Physiol Renal Physiol.* 2005;289(3):F506-11.
133. Burg MB, Ferraris JD, Dmitrieva NI. Cellular Response to Hyperosmotic Stresses. *Physiol Rev.* 2007;87(4):1441-74.
134. Dmitrieva NI, Bulavin DV, Burg MB. High NaCl causes Mre11 to leave the nucleus, disrupting DNA damage signaling and repair. *Am J Physiol Renal Physiol.* 2003;285(2):F266-74.
135. Dmitrieva NI, Cai Q, Burg MB. Cells adapted to high NaCl have many DNA breaks and impaired DNA repair both in cell culture and in vivo. *Proc Natl Acad Sci U S A.* 2004;101(8):2317-22.
136. Stroka KM, Jiang H, Chen S-H, Tong Z, Wirtz D, Sun SX, et al. Water permeation drives tumor cell migration in confined microenvironments. *Cell.* 2014;157(3):611-23.
137. Del Monaco SM, Marino GI, Assef YA, Damiano AE, Kotsias BA. Cell migration in BeWo cells and the role of epithelial sodium channels. *J Membr Biol.* 2009;232(1-3):1-13.
138. Rooj AK, McNicholas CM, Bartoszewski R, Bebok Z, Benos DJ, Fuller CM. Glioma-specific cation conductance regulates migration and cell cycle progression. *J Biol Chem.* 2012;287(6):4053-65.
139. Fraser SP, Ding Y, Liu A, Foster CS, Djamgoz MB. Tetrodotoxin suppresses morphological enhancement of the metastatic MAT-LyLu rat prostate cancer cell line. *Cell Tissue Res.* 1999;295(3):505-12.
140. Brackenbury WJ, Chioni AM, Diss JK, Djamgoz MB. The neonatal splice variant of Nav1.5 potentiates in vitro metastatic behaviour of MDA-MB-231 human breast cancer cells. *Breast Cancer Res Treat.* 2007;101(2):149-60.
141. Brackenbury WJ, Djamgoz MB. Activity-dependent regulation of voltage-gated Na<sup>+</sup> channel expression in Mat-LyLu rat prostate cancer cell line. *J Physiol.* 2006;573(Pt 2):343-56.

142. Brisson L, Driffort V, Benoist L, Poet M, Counillon L, Antelmi E, et al. NaV1.5 Na(+) channels allosterically regulate the NHE-1 exchanger and promote the activity of breast cancer cell invadopodia. *J Cell Sci.* 2013;126(Pt 21):4835-42.
143. Fulgenzi G, Graciotti L, Faronato M, Soldovieri MV, Miceli F, Amoroso S, et al. Human neoplastic mesothelial cells express voltage-gated sodium channels involved in cell motility. *Int J Biochem Cell Biol.* 2006;38(7):1146-59.
144. Gao R, Shen Y, Cai J, Lei M, Wang Z. Expression of voltage-gated sodium channel alpha subunit in human ovarian cancer. *Oncol Rep.* 2010;23(5):1293-9.
145. Song M, Chen D, Yu SP. The TRPC channel blocker SKF 96365 inhibits glioblastoma cell growth by enhancing reverse mode of the Na(+) /Ca(2+) exchanger and increasing intracellular Ca(2+). *Br J Pharmacol.* 2014;171(14):3432-47.
146. Wen J, Pang Y, Zhou T, Qi X, Zhao M, Xuan B, et al. Essential role of Na<sup>+</sup>/Ca<sup>2+</sup> exchanger 1 in smoking-induced growth and migration of esophageal squamous cell carcinoma. *Oncotarget.* 2016;7(39):63816-28.
147. Linck B, Qiu Z, He Z, Tong Q, Hilgemann DW, Philipson KD. Functional comparison of the three isoforms of the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX1, NCX2, NCX3). *Am J Physiol.* 1998;274(2 Pt 1):C415-23.
148. Blaustein MP, Lederer WJ. Sodium/calcium exchange: its physiological implications. *Physiol Rev.* 1999;79(3):763-854.
149. Pappalardo LW, Samad OA, Black JA, Waxman SG. Voltage-gated sodium channel Nav1.5 contributes to astrogliosis in an in vitro model of glial injury via reverse Na<sup>+</sup>/Ca<sup>2+</sup> exchange. *Glia.* 2014;62(7):1162-75.
150. Xu J, Yang Y, Xie R, Liu J, Nie X, An J, et al. The NCX1/TRPC6 Complex Mediates TGFβ-Driven Migration and Invasion of Human Hepatocellular Carcinoma Cells. *Cancer Res.* 2018;78(10):2564-76.
151. Dong H, Shim KN, Li JM, Estrema C, Ornelas TA, Nguyen F, et al. Molecular mechanisms underlying Ca<sup>2+</sup>-mediated motility of human pancreatic duct cells. *Am J Physiol Cell Physiol.* 2010;299(6):C1493-503.
152. Gillet L, Roger S, Besson P, Lecaille F, Gore J, Bougnoux P, et al. Voltage-gated Sodium Channel Activity Promotes Cysteine Cathepsin-dependent Invasiveness and Colony Growth of Human Cancer Cells. *J Biol Chem.* 2009;284(13):8680-91.
153. Marian T, Szabo-Peli J, Nemeth E, Tron L, Friedlander E, Szabo A, et al. Na<sup>+</sup>/Ca<sup>2+</sup> exchanger inhibitors modify the accumulation of tumor-diagnostic PET tracers in cancer cells. *Eur J Pharm Sci.* 2007;30(1):56-63.
154. Turk B, Dolenc I, Lenarcic B, Krizaj I, Turk V, Bieth JG, et al. Acidic pH as a physiological regulator of human cathepsin L activity. *Eur J Biochem.* 1999;259(3):926-32.
155. Magalhaes MAO, Larson DR, Mader CC, Bravo-Cordero JJ, Gil-Henn H, Oser M, et al. Cortactin phosphorylation regulates cell invasion through a pH-dependent pathway. *J Cell Biol.* 2011;195(5):903-20.
156. Busco G, Cardone RA, Greco MR, Bellizzi A, Colella M, Antelmi E, et al. NHE1 promotes invadopodial ECM proteolysis through acidification of the peri-invadopodial space. *FASEB J.* 2010;24(10):3903-15.
157. Greco MR, Antelmi E, Busco G, Guerra L, Rubino R, Casavola V, et al. Protease activity at invadopodial focal digestive areas is dependent on NHE1-driven acidic pH. *Oncol Rep.* 2014;31(2):940-6.
158. Rofstad EK, Mathiesen B, Kindem K, Galappathi K. Acidic extracellular pH promotes experimental metastasis of human melanoma cells in athymic nude mice. *Cancer Res.* 2006;66(13):6699-707.
159. Stock C, Gassner B, Hauck CR, Arnold H, Mally S, Eble JA, et al. Migration of human melanoma cells depends on extracellular pH and Na<sup>+</sup>/H<sup>+</sup> exchange. *J Physiol.* 2005;567(Pt 1):225-38.
160. Fraser SP, Diss JKJ, Chioni AM, Mycielska ME, Pan HY, Yamaci RF, et al. Voltage-gated sodium channel expression and potentiation of human breast cancer metastasis. *Clin Cancer Res.* 2005;11(15):5381-9.

161. House CD, Vaske CJ, Schwartz AM, Obias V, Frank B, Luu T, et al. Voltage-gated Na<sup>+</sup> channel SCN5A is a key regulator of a gene transcriptional network that controls colon cancer invasion. *Cancer Res.* 2010;70(17):6957-67.
162. Driffort V, Gillet L, Bon E, Marionneau-Lambot S, Oullier T, Joulin V, et al. Ranolazine inhibits Nav1.5-mediated breast cancer cell invasiveness and lung colonization. *Mol Cancer.* 2014;13:264.
163. Nelson M, Yang M, Millican-Slater R, Brackenbury WJ. Nav1.5 regulates breast tumor growth and metastatic dissemination in vivo. *Oncotarget.* 2015;6(32):32914-29.
164. House CD, Wang BD, Ceniccola K, Williams R, Simaan M, Olender J, et al. Voltage-gated Na<sup>+</sup> Channel Activity Increases Colon Cancer Transcriptional Activity and Invasion Via Persistent MAPK Signaling. *Sci Rep.* 2015;5:11541.
165. Jin C, Ye Q-H, Yuan F-L, Gu Y-L, Li J-P, Shi Y-H, et al. Involvement of acid-sensing ion channel 1 $\alpha$  in hepatic carcinoma cell migration and invasion. *Tumour Biol.* 2015;36(6):4309-17.
166. Wu Y, Gao B, Xiong Q-J, Wang Y-C, Huang D-K, Wu W-N. Acid-sensing ion channels contribute to the effect of extracellular acidosis on proliferation and migration of A549 cells. *Tumour Biol.* 2017;39(6):1010428317705750.
167. Zhu S, Zhou H-Y, Deng S-C, Deng S-J, He C, Li X, et al. ASIC1 and ASIC3 contribute to acidity-induced EMT of pancreatic cancer through activating Ca/RhoA pathway. *Cell Death Dis.* 2017;8(5):e2806.
168. Haas BR, Sontheimer H. Inhibition of the Sodium-Potassium-Chloride Cotransporter Isoform-1 reduces glioma invasion. *Cancer Res.* 2010;70(13):5597-606.
169. Schliess F, Schafer C, vom Dahl S, Fischer R, Lordnejad MR, Haussinger D. Expression and regulation of the Na<sup>(+)</sup>/K<sup>(+)</sup>/2Cl<sup>(-)</sup> cotransporter NKCC1 in rat liver and human HuH-7 hepatoma cells. *Arch Biochem Biophys.* 2002;401(2):187-97.
170. Jantsch J, Schatz V, Friedrich D, Schroder A, Kopp C, Siebert I, et al. Cutaneous Na<sup>+</sup> storage strengthens the antimicrobial barrier function of the skin and boosts macrophage-driven host defense. *Cell Metab.* 2015;21(3):493-501.
171. Schwartz L, Guais A, Pooya M, Abolhassani M. Is inflammation a consequence of extracellular hyperosmolarity? *J Inflamm.* 2009;6:21.
172. Zhang W-C, Zheng X-J, Du L-J, Sun J-Y, Shen Z-X, Shi C, et al. High salt primes a specific activation state of macrophages, M(Na). *Cell Res.* 2015;25(8):893-910.
173. Wu C, Yosef N, Thalhamer T, Zhu C, Xiao S, Kishi Y, et al. Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. *Nature.* 2013;496(7446):513-7.
174. Coussens LM, Werb Z. Inflammation and cancer. *Nature.* 2002;420(6917):860-7.
175. Németh ZH, Deitch EA, Szabó C, Haskó G. Hyperosmotic Stress Induces Nuclear Factor- $\kappa$ B Activation and Interleukin-8 Production in Human Intestinal Epithelial Cells. *Am J Pathol.* 2002;161(3):987-96.
176. Shanfield S, Campbell P, Baumgarten M, Bloebaum R, Sarmiento A. Synovial fluid osmolality in osteoarthritis and rheumatoid arthritis. *Clin Orthop Relat Res.* 1988(235):289-95.
177. Kino T, Takatori H, Manoli I, Wang Y, Tiulpakov A, Blackman MR, et al. Brx mediates the response of lymphocytes to osmotic stress through the activation of NFAT5. *Sci Signal.* 2009;2(57):ra5.
178. Dmitrieva NI, Burg MB. Elevated sodium and dehydration stimulate inflammatory signaling in endothelial cells and promote atherosclerosis. *PLoS One.* 2015;10(6):e0128870.
179. Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K, et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med.* 2009;15(5):545-52.
180. Shapiro L, Dinarello CA. Osmotic regulation of cytokine synthesis in vitro. *Proceedings of the National Academy of Sciences.* 1995;92(26):12230-4.
181. Allavena P, Mantovani A. Immunology in the clinic review series; focus on cancer: tumour-associated macrophages: undisputed stars of the inflammatory tumour microenvironment. *Clin Exp Immunol.* 2012;167(2):195-205.



182. Amara S, Whalen M, Tiriveedhi V. High salt induces anti-inflammatory MΦ2-like phenotype in peripheral macrophages. *Biochem Biophys Rep.* 2016;7:1-9.
183. Amara S, Tiriveedhi V. Inflammatory role of high salt level in tumor microenvironment. *Int J Oncol.* 2017;50(5):1477-81.
184. Tartour E, Fossiez F, Joyeux I, Galinha A, Gey A, Claret E, et al. Interleukin 17, a T-cell-derived cytokine, promotes tumorigenicity of human cervical tumors in nude mice. *Cancer Res.* 1999;59(15):3698-704.
185. Ernst M, Putoczki T. IL-17 cuts to the chase in colon cancer. *Immunity.* 2014;41(6):880-2.
186. Numasaki M, Watanabe M, Suzuki T, Takahashi H, Nakamura A, McAllister F, et al. IL-17 enhances the net angiogenic activity and in vivo growth of human non-small cell lung cancer in SCID mice through promoting CXCR-2-dependent angiogenesis. *J Immunol.* 2005;175(9):6177-89.
187. Amara S, Alotaibi D, Tiriveedhi V. NFAT5/STAT3 interaction mediates synergism of high salt with IL-17 towards induction of VEGF-A expression in breast cancer cells. *Oncol Lett.* 2016;12(2):933-43.
188. Babaer D, Amara S, Ivy M, Zhao Y, Lammers PE, Titze JM, et al. High salt induces P-glycoprotein mediated treatment resistance in breast cancer cells through store operated calcium influx. *Oncotarget.* 2018;9(38):25193-205.
189. Jacobs MA, Ouwerkerk R, Wolff AC, Gabrielson E, Warzecha H, Jeter S, et al. Monitoring of neoadjuvant chemotherapy using multiparametric, <sup>23</sup>Na sodium MR, and multimodality (PET/CT/MRI) imaging in locally advanced breast cancer. *Breast Cancer Res Treat.* 2011;128(1):119-26.
190. Schuier G, Ladebeck R, Barfuß H, Hentschel D, Huk WJ. Sodium-23 imaging of supratentorial lesions at 4.0 T. *Magn Reson Med.* 1991;22(1):1-9.
191. Jacobs MA, Ouwerkerk R, Wolff AC, Stearns V, Bottomley PA, Barker PB, et al. Multiparametric and multinuclear magnetic resonance imaging of human breast cancer: current applications. *Technol Cancer Res Treat.* 2004;3(6):543-50.
192. Nagel AM, Bock M, Hartmann C, Gerigk L, Neumann JO, Weber MA, et al. The potential of relaxation-weighted sodium magnetic resonance imaging as demonstrated on brain tumors. *Invest Radiol.* 2011;46(9):539-47.
193. Zaric O, Pinker K, Zbyn S, Strasser B, Robinson S, Minarikova L, et al. Quantitative Sodium MR Imaging at 7 T: Initial Results and Comparison with Diffusion-weighted Imaging in Patients with Breast Tumors. *Radiology.* 2016;280(1):39-48.
194. Nunes Neto LP, Madelin G, Sood TP, Wu CC, Kondziolka D, Placantonakis D, et al. Quantitative sodium imaging and gliomas: a feasibility study. *Neuroradiology.* 2018;60(8):795-802.
195. Madelin G, Kline R, Walvick R, Regatte RR. A method for estimating intracellular sodium concentration and extracellular volume fraction in brain in vivo using sodium magnetic resonance imaging. *Sci Rep.* 2014;4:4763.
196. Laymon CM, Oborski MJ, Lee VK, Davis DK, Wiener EC, Lieberman FS, et al. Combined imaging biomarkers for therapy evaluation in glioblastoma multiforme: Correlating sodium MRI and F-18 FLT PET on a voxel-wise basis. *Magn Reson Imaging.* 2012;30(9):1268-78.
197. Biller A, Badde S, Nagel A, Neumann JO, Wick W, Hertenstein A, et al. Improved Brain Tumor Classification by Sodium MR Imaging: Prediction of IDH Mutation Status and Tumor Progression. *AJNR Am J Neuroradiol.* 2016;37(1):66-73.
198. Thulborn KR, Lu A, Atkinson IC, Pauliah M, Beal K, Chan TA, et al. Residual Tumor Volume, Cell Volume Fraction and Tumor Cell Kill During Fractionated Chemoradiation Therapy of Human Glioblastoma using Quantitative Sodium MR imaging. *Clin Cancer Res.* 2018(23):clincanres.2079.18.
199. Winter PM, Poptani H, Bansal N. Effects of chemotherapy by 1,3-bis(2-chloroethyl)-1-nitrosourea on single-quantum- and triple-quantum-filtered <sup>23</sup>Na and <sup>31</sup>P nuclear magnetic resonance of the subcutaneously implanted 9L glioma. *Cancer Res.* 2001;61(5):2002-7.

200. Jacobs MA, Stearns V, Wolff AC, Macura K, Argani P, Khouri N, et al. Multiparametric Magnetic Resonance Imaging, Spectroscopy and Multinuclear ( $^{23}\text{Na}$ ) Imaging Monitoring of Preoperative Chemotherapy for Locally Advanced Breast Cancer. *Acad Radiol*. 2010;17(12):1477-85.
201. Schepkin VD, Bejarano FC, Morgan T, Gower-Winter S, Ozambela M, Jr., Levenson CW. In vivo magnetic resonance imaging of sodium and diffusion in rat glioma at 21.1 T. *Magn Reson Med*. 2012;67(4):1159-66.
202. Babsky AM, Hekmatyar SK, Zhang H, Solomon JL, Bansal N. Application of  $^{23}\text{Na}$  MRI to monitor chemotherapeutic response in RIF-1 tumors. *Neoplasia*. 2005;7(7):658-66.
203. Barthä R, Megyesi JF, Watling CJ. Low-grade glioma: correlation of short echo time  $^1\text{H}$ -MR spectroscopy with  $^{23}\text{Na}$  MR imaging. *AJNR Am J Neuroradiol*. 2008;29(3):464-70.
204. Taylor S, Spugnini EP, Assaraf YG, Azzarito T, Rauch C, Fais S. Microenvironment acidity as a major determinant of tumor chemoresistance: Proton pump inhibitors (PPIs) as a novel therapeutic approach. *Drug Resist Updat*. 2015;23:69-78.
205. Poon AC, Inkol JM, Luu AK, Mutsaers AJ. Effects of the potassium-sparing diuretic amiloride on chemotherapy response in canine osteosarcoma cells. *J Vet Intern Med*. 2019;33(2):800-11.
206. Jiang W, Li G, Li W, Wang P, Xiu P, Jiang X, et al. Sodium orthovanadate overcomes sorafenib resistance of hepatocellular carcinoma cells by inhibiting Na/K-ATPase activity and hypoxia-inducible pathways. *Sci Rep*. 2018;8(1):9706.
207. Lardner A. The effects of extracellular pH on immune function. *J Leukoc Biol*. 2001;69(4):522-30.
208. Loeffler DA, Juneau PL, Heppner GH. Natural killer-cell activity under conditions reflective of tumor micro-environment. *Int J Cancer*. 1991;48(6):895-9.
209. Redegeld F, Filippini A, Sitkovsky M. Comparative studies of the cytotoxic T lymphocyte-mediated cytotoxicity and of extracellular ATP-induced cell lysis. Different requirements in extracellular  $\text{Mg}^{2+}$  and pH. *J Immunol*. 1991;147(10):3638-45.
210. Goldman S, Bouffet E, Fisher PG, Allen JC, Robertson PL, Chuba PJ, et al. Phase II Trial Assessing the Ability of Neoadjuvant Chemotherapy With or Without Second-Look Surgery to Eliminate Measurable Disease for Nongerminomatous Germ Cell Tumors: A Children's Oncology Group Study. *J Clin Oncol*. 2015;33(22):2464.
211. Ghadimi K, Gutsche JT, Ramakrishna H, Setegne SL, Jackson KR, Augoustides JG, et al. Sodium bicarbonate use and the risk of hypernatremia in thoracic aortic surgical patients with metabolic acidosis following deep hypothermic circulatory arrest. *Ann Card Anaesth*. 2016;19(3):454.
212. Ibrahim-Hashim A, Abrahams D, Enriquez-Navas PM, Luddy K, Gatenby RA, Gillies RJ. Tris-base buffer: a promising new inhibitor for cancer progression and metastasis. *Cancer Med*. 2017;6(7):1720-9.
213. Nelson M, Yang M, Dowle AA, Thomas JR, Brackenbury WJ. The sodium channel-blocking antiepileptic drug phenytoin inhibits breast tumour growth and metastasis. *Mol Cancer*. 2015;14(1):13.
214. Martin F, Ufodiama C, Watt I, Bland M, Brackenbury WJ. Therapeutic value of voltage-gated sodium channel inhibitors in breast, colorectal and prostate cancer: a systematic review. *Front Pharmacol*. 2015;6:273.
215. Dutta S, Lopez Charcas O, Tanner S, Gradek F, Driffort V, Roger S, et al. Discovery and evaluation of  $\text{nNa}1.5$  sodium channel blockers with potent cell invasion inhibitory activity in breast cancer cells. *Bioorg Med Chem*. 2018;26(9):2428-36.
216. Walker AJ, Card T, Bates TE, Muir K. Tricyclic antidepressants and the incidence of certain cancers: a study using the GPRD. *Br J Cancer*. 2011;104(1):193-7.
217. Takada M, Fujimoto M, Motomura H, Hosomi K. Inverse Association between Sodium Channel-Blocking Antiepileptic Drug Use and Cancer: Data Mining of Spontaneous Reporting and Claims Databases. *Int J Med Sci*. 2016;13(1):48-59.
218. Fairhurst C, Watt I, Martin F, Bland M, Brackenbury WJ. Exposure to sodium channel-inhibiting drugs and cancer survival: protocol for a cohort study using the QResearch primary care database. *BMJ Open*. 2014;4(11):e006604.

219. Fairhurst C, Watt I, Martin F, Bland M, Brackenbury WJ. Sodium channel-inhibiting drugs and survival of breast, colon and prostate cancer: a population-based study. *Sci Rep*. 2015;5:16758.
220. Exadaktylos AK, Buggy DJ, Moriarty DC, Mascha E, Sessler DI. Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? *Anesthesiology*. 2006;105(4):660-4.
221. Scherpereel A, Berghmans T, Lafitte JJ, Colinet B, Richez M, Bonduelle Y, et al. Valproate-doxorubicin: promising therapy for progressing mesothelioma. A phase II study. *Eur Respir J*. 2011;37(1):129-35.
222. Daud AI, Dawson J, DeConti RC, Bicaku E, Marchion D, Bastien S, et al. Potentiation of a topoisomerase I inhibitor, karenitecin, by the histone deacetylase inhibitor valproic acid in melanoma: translational and phase I/II clinical trial. *Clin Cancer Res*. 2009;15(7):2479-87.
223. Bharati SJ, Chowdhury T, Bergese SD, Ghosh S. Anesthetics impact on cancer recurrence: What do we know? *J Cancer Res Ther*. 2016;12(2):464-8.
224. Cata JP, Ramirez MF, Velasquez JF, Di AI, Popat KU, Gottumukkala V, et al. Lidocaine Stimulates the Function of Natural Killer Cells in Different Experimental Settings. *Anticancer Res*. 2017;37(9):4727-32.
225. Piegeler T, Votta-Velis EG, Liu G, Place AT, Schwartz DE, Beck-Schimmer B, et al. Antimetastatic potential of amide-linked local anesthetics: inhibition of lung adenocarcinoma cell migration and inflammatory Src signaling independent of sodium channel blockade. *Anesthesiology*. 2012;117(3):548-59.
226. Brodie SA, Brandes JC. Could valproic acid be an effective anticancer agent? The evidence so far. *Expert Rev Anticancer Ther*. 2014;14(10):1097-100.
227. Isbilen B, Fraser SP, Djamgoz MBA. Docosahexaenoic acid (omega-3) blocks voltage-gated sodium channel activity and migration of MDA-MB-231 human breast cancer cells. *Int J Biochem Cell Biol*. 2006;38(12):2173-82.
228. Gillet L, Roger S, Bougnoux P, Le Guennec J-Y, Besson P. Beneficial effects of omega-3 long-chain fatty acids in breast cancer and cardiovascular diseases: voltage-gated sodium channels as a common feature? *Biochimie*. 2011;93(1):4-6.
229. Djamgoz MBA. Blood pressure and risk of cancer progression – A possible connection with salt and voltage-gated sodium channel. *Med Hypotheses*. 2015;85(5):591-3.
230. D'Elia L, Rossi G, Ippolito R, Cappuccio FP, Strazzullo P. Habitual salt intake and risk of gastric cancer: a meta-analysis of prospective studies. *Clin Nutr*. 2012;31(4):489-98.
231. Lo WL, Donermeyer DL, Allen PM. A voltage-gated sodium channel is essential for the positive selection of CD4(+) T cells. *Nat Immunol*. 2012;13(9):880-7.
232. Bortner CD, Cidlowski JA. Uncoupling cell shrinkage from apoptosis reveals that Na<sup>+</sup> influx is required for volume loss during programmed cell death. *J Biol Chem*. 2003;278(40):39176-84.
233. Tannous BA, Christensen AP, Pike L, Wurdinger T, Perry KF, Saydam O, et al. Mutant sodium channel for tumor therapy. *Mol Ther*. 2009;17(5):810-9.
234. Gould HJ, 3rd, Norleans J, Ward TD, Reid C, Paul D. Selective lysis of breast carcinomas by simultaneous stimulation of sodium channels and blockade of sodium pumps. *Oncotarget*. 2018;9(21):15606-15.
235. Kepp O, Menger L, Vacchelli E, Adjemian S, Martins I, Ma Y, et al. Anticancer activity of cardiac glycosides: At the frontier between cell-autonomous and immunological effects. *Oncoimmunology*. 2012;1(9):1640-2.
236. Menger L, Vacchelli E, Adjemian S, Martins I, Ma Y, Shen S, et al. Cardiac glycosides exert anticancer effects by inducing immunogenic cell death. *Sci Transl Med*. 2012;4(143):143ra99.
237. Li L, Feng R, Xu Q, Zhang F, Liu T, Cao J, et al. Expression of the beta3 subunit of Na<sup>+</sup>/K<sup>+</sup>-ATPase is increased in gastric cancer and regulates gastric cancer cell progression and prognosis via the PI3/AKT pathway. *Oncotarget*. 2017;8(48):84285-99.
238. Kapoor N, Bartoszewski R, Qadri YJ, Bebok Z, Bubien JK, Fuller CM, et al. Knockdown of ASIC1 and epithelial sodium channel subunits inhibits glioblastoma whole cell current and cell migration. *J Biol Chem*. 2009;284(36):24526-41.

239. Yamamura H, Ugawa S, Ueda T, Shimada S. Expression analysis of the epithelial Na<sup>+</sup> channel delta subunit in human melanoma G-361 cells. *Biochem Biophys Res Commun.* 2008;366(2):489-92.
240. Ye JH, Gao J, Wu YN, Hu YJ, Zhang CP, Xu TL. Identification of acid-sensing ion channels in adenoid cystic carcinomas. *Biochem Biophys Res Commun.* 2007;355(4):986-92.
241. Blandino JK, Viglione MP, Bradley WA, Oie HK, Kim YI. Voltage-dependent sodium channels in human small-cell lung cancer cells: role in action potentials and inhibition by Lambert-Eaton syndrome IgG. *J Membr Biol.* 1995;143(2):153-63.
242. Laniado ME, Lalani EN, Fraser SP, Grimes JA, Bhangal G, Djamgoz MB, et al. Expression and functional analysis of voltage-activated Na<sup>+</sup> channels in human prostate cancer cell lines and their contribution to invasion in vitro. *Am J Pathol.* 1997;150(4):1213-21.
243. Diaz D, Delgadillo DM, Hernandez-Gallegos E, Ramirez-Dominguez ME, Hinojosa LM, Ortiz CS, et al. Functional expression of voltage-gated sodium channels in primary cultures of human cervical cancer. *J Cell Physiol.* 2007;210(2):469-78.
244. Zhou Y, Sun W, Chen N, Xu C, Wang X, Dong K, et al. Discovery of NKCC1 as a potential therapeutic target to inhibit hepatocellular carcinoma cell growth and metastasis. *Oncotarget.* 2017;8(39):66328-42.
245. Sun PL, Jin Y, Park SY, Kim H, Park E, Jheon S, et al. Expression of Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter isoform 1 (NKCC1) predicts poor prognosis in lung adenocarcinoma and EGFR-mutated adenocarcinoma patients. *Qjm.* 2016;109(4):237-44.
246. Deutsch SI, Tang AH, Burket JA, Benson AD. NMDA receptors on the surface of cancer cells: target for chemotherapy? *Biomed Pharmacother.* 2014;68(4):493-6.
247. McLean LA, Roscoe J, Jorgensen NK, Gorin FA, Cala PM. Malignant gliomas display altered pH regulation by NHE1 compared with nontransformed astrocytes. *Am J Physiol Cell Physiol.* 2000;278(4):C676-88.
248. Kaminota T, Yano H, Shiota K, Nomura N, Yaguchi H, Kirino Y, et al. Elevated Na<sup>+</sup>/H<sup>+</sup> exchanger-1 expression enhances the metastatic collective migration of head and neck squamous cell carcinoma cells. *Biochem Biophys Res Commun.* 2017;486(1):101-7.
249. Amith SR, Vincent KM, Wilkinson JM, Postovit LM, Fliegel L. Defining the Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1 interactome in triple-negative breast cancer cells. *Cell Signal.* 2017;29:69-77.
250. Yang X, Wang D, Dong W, Song Z, Dou K. Expression and modulation of Na<sup>+</sup>/H<sup>+</sup> exchanger 1 gene in hepatocellular carcinoma: A potential therapeutic target. *J Gastroenterol Hepatol.* 2011;26(2):364-70.
251. Lee S, Mele M, Vahl P, Christiansen PM, Jensen VE, Boedtkjer E. Na<sup>+</sup>,HCO<sub>3</sub><sup>-</sup> cotransport is functionally upregulated during human breast carcinogenesis and required for the inverted pH gradient across the plasma membrane. *Pflugers Arch.* 2015;467(2):367-77.
252. Ahmed S, Thomas G, Ghoussaini M, Healey CS, Humphreys MK, Platte R, et al. Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat Genet.* 2009;41(5):585-90.
253. Hassanein M, Qian J, Hoeksema MD, Wang J, Jacobovitz M, Ji X, et al. Targeting SLC1a5-mediated glutamine dependence in non-small cell lung cancer. *Int J Cancer.* 2015;137(7):1587-97.
254. van Geldermalsen M, Wang Q, Nagarajah R, Marshall AD, Thoeng A, Gao D, et al. ASCT2/SLC1A5 controls glutamine uptake and tumour growth in triple-negative basal-like breast cancer. *Oncogene.* 2016;35(24):3201-8.
255. Fuchs BC, Bode BP. Amino acid transporters ASCT2 and LAT1 in cancer: partners in crime? *Semin Cancer Biol.* 2005;15(4):254-66.
256. Witte D, Ali N, Carlson N, Younes M. Overexpression of the neutral amino acid transporter ASCT2 in human colorectal adenocarcinoma. *Anticancer Res.* 2002;22(5):2555-7.

257. Dolinska M, Dybel A, Zablocka B, Albrecht J. Glutamine transport in C6 glioma cells shows ASCT2 system characteristics. *Neurochem Int.* 2003;43(4-5):501-7.
258. Gupta N, Miyauchi S, Martindale RG, Herdman AV, Podolsky R, Miyake K, et al. Upregulation of the amino acid transporter ATB<sub>0,+</sub> (SLC6A14) in colorectal cancer and metastasis in humans. *Biochim Biophys Acta.* 2005;1741(1-2):215-23.
259. Gupta N, Prasad PD, Ghamande S, Moore-Martin P, Herdman AV, Martindale RG, et al. Up-regulation of the amino acid transporter ATB<sub>0,+</sub> (SLC6A14) in carcinoma of the cervix. *Gynecol Oncol.* 2006;100(1):8-13.
260. Sidoryk M, Matyja E, Dybel A, Zielinska M, Bogucki J, Jaskolski DJ, et al. Increased expression of a glutamine transporter SNAT3 is a marker of malignant gliomas. *Neuroreport.* 2004;15(4):575-8.
261. Kondoh N, Imazeki N, Arai M, Hada A, Hatsuse K, Matsuo H, et al. Activation of a system A amino acid transporter, ATA1/SLC38A1, in human hepatocellular carcinoma and preneoplastic liver tissues. *Int J Oncol.* 2007;31(1):81-7.
262. Yu WL, Cong WM, Zhang Y, Chen Y, Wang F, Yu G. Overexpression of ATA1/SLC38A1 predicts future recurrence and death in Chinese patients with hilar cholangiocarcinoma. *J Surg Res.* 2011;171(2):663-8.
263. Balasubramaniam SL, Gopalakrishnapillai A, Petrelli NJ, Barwe SP. Knockdown of sodium-calcium exchanger 1 induces epithelial-to-mesenchymal transition in kidney epithelial cells. *J Biol Chem.* 2017;292(27):11388-99.
264. Matthews H, Ranson M, Kelso MJ. Anti-tumour/metastasis effects of the potassium-sparing diuretic amiloride: an orally active anti-cancer drug waiting for its call-of-duty? *Int J Cancer.* 2011;129(9):2051-61.
265. Mentzer RM, Jr., Bartels C, Bolli R, Boyce S, Buckberg GD, Chaitman B, et al. Sodium-hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery bypass grafting: results of the EXPEDITION study. *Ann Thorac Surg.* 2008;85(4):1261-70.
266. Terkawi AS, Durieux ME, Gottschalk A, Brenin D, Tiouririne M. Effect of intravenous lidocaine on postoperative recovery of patients undergoing mastectomy: a double-blind, placebo-controlled randomized trial. *Reg Anesth Pain Med.* 2014;39(6):472-7.
267. Christopherson R, James KE, Tableman M, Marshall P, Johnson FE. Long-term survival after colon cancer surgery: a variation associated with choice of anesthesia. *Anesth Analg.* 2008;107(1):325-32.
268. Coronel J, Cetina L, Pacheco I, Trejo-Becerril C, Gonzalez-Fierro A, de la Cruz-Hernandez E, et al. A double-blind, placebo-controlled, randomized phase III trial of chemotherapy plus epigenetic therapy with hydralazine valproate for advanced cervical cancer. Preliminary results. *Med Oncol.* 2011;28 Suppl 1:S540-6.
269. Nilubol N, Merkel R, Yang L, Patel D, Reynolds JC, Sadowski SM, et al. A phase II trial of valproic acid in patients with advanced, radioiodine-resistant thyroid cancers of follicular cell origin. *Clin Endocrinol (Oxf).* 2017;86(1):128-33.
270. Berghmans T, Lafitte JJ, Scherpereel A, Ameye L, Paesmans M, Meert AP, et al. VAC chemotherapy with valproic acid for refractory/relapsing small cell lung cancer: a phase II study. *ERJ Open Res.* 2015;1(2).
271. Lin J, Zhan T, Duffy D, Hoffman-Censits J, Kilpatrick D, Trabulsi EJ, et al. A pilot phase II Study of digoxin in patients with recurrent prostate cancer as evident by a rising PSA. *Am J Cancer Ther Pharmacol.* 2014;2(1):21-32.
272. North WG, Gao G, Memoli VA, Pang RH, Lynch L. Breast cancer expresses functional NMDA receptors. *Breast Cancer Res Treat.* 2010;122(2):307-14.
273. Long Z, Chen B, Liu Q, Zhao J, Yang Z, Dong X, et al. The reverse-mode NCX1 activity inhibitor KB-R7943 promotes prostate cancer cell death by activating the JNK pathway and blocking autophagic flux. *Oncotarget.* 2016;7(27):42059-70.
274. Parrish TB, Fieno DS, Fitzgerald SW, Judd RM. Theoretical basis for sodium and potassium MRI of the human heart at 1.5 T. *Magn Reson Med.* 1997;38(4):653-61.
275. Maudsley AAA, Hilal SKK. Biological Aspects of Sodium-23 Imaging. *Br Med Bull.* 1984;40:165-6.

276. Hilal SK, Maudsley AA, Ra JB, Simon HE, Roschmann P, Wittekoek S, et al. In vivo NMR imaging of sodium-23 in the human head. *J Comput Assist Tomogr.* 1985;9(1):1-7.
277. Feinberg DA, Crooks LA, Kaufman L, Brant-Zawadzki M, Posin JP, Arakawa M, et al. Magnetic resonance imaging performance: a comparison of sodium and hydrogen. *Radiology.* 1985;156(1):133-8.
278. Oh CH, Hilal SK, Ra JB, Mun JK, Cho ZH. Gradient recalled echo sodium magnetic resonance by using plane integral projection reconstruction. *Proc Int Soc Magn Reson Med Sci Meet Exhib Int Soc Magn Reson Med Sci Meet Exhib.* 1987:904.
279. Winkler SS, Thomasson DM, Sherwood K, Perman WH. Regional T2 and Sodium Concentration Estimates in the Normal Human Brain by Sodium-23 MR Imaging at 1.5 T. *J Comput Assist Tomogr.* 1989;13(4):561-6.
280. Turski PA, Houston LW, Perman WH, Hald JK, Turski D, Strother CM, et al. Experimental and human brain neoplasms: detection with in vivo sodium MR imaging. *Radiology.* 1987;163(1):245-9.
281. Hashimoto T, Ikehira H, Fukuda H, Yamaura A, Watanabe O, Tateno Y, et al. In vivo sodium-23 MRI in brain tumors: evaluation of preliminary clinical experience. *Am J Physiol Imaging.* 1991;6(2):74-80.
282. Christensen JD, Barrere BJ, Boada FE, Vevea JM, Thulborn KR. Quantitative Tissue Sodium Concentration Mapping of Normal Rat Brain. *Magn Reson Med.* 1996;36:83-9.
283. Boada FE, Christensen JD, Huang-Hellinger FR, Reese TG, Thulborn KR. Quantitative in vivo tissue sodium concentration maps: The effects of biexponential relaxation. *Magn Reson Med.* 1994;32(2):219-23.
284. Boada FE, Gillen JS, Shen GX, Chang SY, Thulborn KR. Fast three dimensional sodium imaging. *Magn Reson Med.* 1997;37(5):706-15.
285. Thulborn KR, Davis D, Adams H, Gindin T, Zhou J. Quantitative tissue sodium concentration mapping of the growth of focal cerebral tumors with sodium magnetic resonance imaging. *Magn Reson Med.* 1999;41(2):351-9.
286. Nagel AM, Bock M, Hartmann C, Gerigk L, Neumann J-O, Weber M-A, et al. The potential of relaxation-weighted sodium magnetic resonance imaging as demonstrated on brain tumors. *Invest Radiol.* 2011;46(9):539-47.
287. Titze J, Shakibaei M, Schafflhuber M, Schulze-Tanzil G, Porst M, Schwind KH, et al. Glycosaminoglycan polymerization may enable osmotically inactive Na<sup>+</sup> storage in the skin. *Am J Physiol Heart Circ Physiol.* 2004;287(1):H203-8.
288. Pedersen SF, Hoffmann EK, Novak I. Cell volume regulation in epithelial physiology and cancer. *Front Physiol.* 2013;4:233.
289. Kahle KT, Khanna AR, Alper SL, Adragna NC, Lauf PK, Sun D, et al. K-Cl cotransporters, cell volume homeostasis, and neurological disease. *Trends Mol Med.* 2015;21(8):513-23.
290. Demaurex N, Grinstein S. Na<sup>+</sup>/H<sup>+</sup> antiport: modulation by ATP and role in cell volume regulation. *J Exp Biol.* 1994;196:389-404.
291. Brackenbury WJ, Isom LL. Na Channel  $\beta$  Subunits: Overachievers of the Ion Channel Family. *Front Pharmacol.* 2011;2:53.
292. Schiapparelli P, Guerrero-Cazares H, Magana-Maldonado R, Hamilla SM, Ganaha S, Goulin Lippi Fernandes E, et al. NKCC1 Regulates Migration Ability of Glioblastoma Cells by Modulation of Actin Dynamics and Interacting with Cofilin. *EBioMedicine.* 2017;21:94-103.
293. Boroughs LK, DeBerardinis RJ. Metabolic pathways promoting cancer cell survival and growth. *Nat Cell Biol.* 2015;17(4):351-9.
294. Amara S, Ivy MT, Myles EL, Tiriveedhi V. Sodium channel  $\gamma$ ENaC mediates IL-17 synergized high salt induced inflammatory stress in breast cancer cells. *Cell Immunol.* 2016;302:1-10.

**Table 1.** Na<sup>+</sup> transport mechanisms with altered expression in cancer.

Transporter	Subtype	Cancer	Change	Effects	References
Na <sup>+</sup> /K <sup>+</sup> ATPase	α3 subunit	Liver, gastric	↑	↑ proliferation ↑ migration ↑ invasion ↓ apoptosis	(206, 237)
ENaC	αENaC, γENaC	Glioblastoma, HCC, melanoma, placenta	↑	↑ migration ↑ proliferation	(121, 137, 238, 239)
ASIC	ASIC1, ASIC1a, ASIC2, ASIC2a, ASIC3	Liver, glioblastoma, PDAC, colorectal, adenoid	↑	↑ invasion ↑ migration ↑ EMT	(56, 165, 167, 238, 240)
VGSC	Na <sub>v</sub> 1.2, Na <sub>v</sub> 1.4, Na <sub>v</sub> 1.5, Na <sub>v</sub> 1.6, Na <sub>v</sub> 1.7	NSCLC, prostate, cervical, colorectal, breast, ovarian	↑	↑ invasion ↑ migration	(26, 140, 144, 160, 161, 241-243)
NKCC	NKCC1	HCC, glioblastoma, NSCLC	↑	↑ invasion ↑ migration ↑ proliferation	(47, 244, 245)
NMDA-R	NMDAR2B	Glioblastoma NSCLC ESCC gastric colorectal breast	↓/↑	↓ proliferation/ ↑ proliferation	(246)
Na <sup>+</sup> /H <sup>+</sup> Exchanger	NHE1	Glioma, HNSCC, breast, HCC, cervical	↑	↑ acid extrusion ↑ invasion	(247-250)
Na <sup>+</sup> /HCO <sub>3</sub> <sup>-</sup> transporter	NBCn1	Breast	↑	↑ acid extrusion	(42, 251, 252)
Na <sup>+</sup> /glucose cotransporter	SGLT2	Breast	↑	↑ glucose uptake?	(46)
Amino acid transporters	SLC1a5/ASCT2	NSCLC, glioblastoma, eye, kidney, liver, lymph node, breast, muscle, placenta, skin, gastric, colorectal	↑	↑ amino acid/glutamine metabolism	(253-257)
	SLC6A14	Cervical, colon, PDAC, breast	↑	↑ amino acid uptake?	(114, 258, 259)
	SLC38a3/SNAT3	Glioma	↑	↑ amino acid uptake?	(260)
	SLC38a1/SNAT1	HCC, bile duct	↑	↑ growth ↑ survival	(261, 262)
NCX		Kidney	↓	↓ EMT	(263)

Abbreviations: ENaC, epithelial Na<sup>+</sup> channel; VGSC, voltage-gated Na<sup>+</sup> channel; NKCC, Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> co-transporter; NMDA-R, N-methyl D-aspartate receptor; NCX, sodium calcium exchanger; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; ESCC, oesophageal squamous cell carcinoma; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma, EMT, epithelial-mesenchymal transition.



**Table 2.** Na<sup>+</sup>-regulating mechanisms as potential therapeutic targets<sup>1</sup>.

Transporter	Compound	Category	Cancer types	Highest phase	Clinical trial NCT numbers and references
ENaC	Amiloride	ENaC inhibitor	Solid tumours	Preclinical	(264)
NHE1	Cariporide	NHE1 inhibitor	NA	Phase III (stopped)	(265)
VGSC	Lidocaine	VGSC blocker, local anaesthetic	Breast, colorectal	Phase III	NCT01916317, NCT02786329, NCT01841294, NCT02839668, NCT02647385, (266)
	Ropivacaine	VGSC blocker, local anaesthetic	Abdominal/thoracic	NA	NCT03134430
	Bupivacaine	VGSC blocker, local anaesthetic	Breast, colon	Phase III	NCT00938171, (267)
	Valproate	VGSC blocker, HDAC inhibitor, antiepileptic	HNSCC, cervical, melanoma, mesothelioma, bladder, thyroid, NSCLC	Phase III	NCT01695122, NCT01738815, (222, 268-270)
	Phenytoin	VGSC blocker, antiepileptic	Breast	Preclinical	(213)
	Ranolazine	VGSC blocker, antianginal	Breast	Preclinical	(162)
Na <sup>+</sup> /K <sup>+</sup> ATPase	Digoxin	Cardiac glycoside, Na <sup>+</sup> /K <sup>+</sup> ATPase inhibitor	Prostate, breast, melanoma, acute myeloid leukaemia/myelodysplastic syndromes, HNSCC	Phase II	NCT02138292, NCT03113071, NCT02906800, NCT01763931, NCT01887288, (221, 271)

	Ouabain	Cardiac glycoside, Na <sup>+</sup> /K <sup>+</sup> ATPase inhibitor	Breast	Preclinical	(234)
NKCC	Bumetanide	NKCC1 inhibitor	HCC	Preclinical	(244)
NMDA-R	Memantine & MK-801	NMDA-R blocker	Breast	Preclinical	(272)
NCX	KB-R7943	NCX reverse mode inhibitor	Prostate	Preclinical	(273)
SGLT2	Empagliflozin and others	SGLT2 inhibitors	Urinary tract	Observational	NCT03464045
N/A	NaHCO <sub>3</sub>	Neutral pH buffer	Any	Phase I	NCT02531919

<sup>1</sup>ENaC, epithelial Na<sup>+</sup> channel; VGSC, voltage-gated Na<sup>+</sup> channel; NKCC, Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> co-transporter; NMDA-R, N-methyl D-aspartate receptor; NCX, sodium calcium exchanger; HDAC, histone deacetylase; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; EMT, epithelial-mesenchymal transition.

### Box 1: Development of $^{23}\text{Na}$ MRI

$^{23}\text{Na}$  MRI is non-invasive and presents a unique mechanism for measuring  $\text{Na}^+$  in tissue.

$^{23}\text{Na}$  MRI does not disturb the tissue state, unlike  $\text{Na}^+$  measurements that require physical tissue sampling.  $\text{Na}^+$  is endogenous to human tissue, which enables imaging without external contrast.  $^{23}\text{Na}$  MRI can differentiate intracellular  $\text{Na}^+$  from total tissue  $\text{Na}^+$ . The non-invasive quantification of cancer  $\text{Na}^+$  content with MRI has the potential to provide a large amount of information on tissue microstructure and function that could improve our understanding of the changes occurring in this disease both early on in formation and in response to therapy.  $^{23}\text{Na}$  MRI is currently primarily used for research due to the non-standard hardware necessary to enable  $\text{Na}^+$  signal acquisition. The signal from  $^{23}\text{Na}$  MRI is much lower than the signal in conventionally used  $\text{H}^+$  MRI for several reasons:  $\text{Na}^+$  has a lower abundance in the human body compared to  $\text{H}^+$ , which proportionally reduces the available magnetization and therefore signal-to-noise; the gyromagnetic ratio of  $\text{Na}^+$  (11.26 MHz/Tesla) is 4 times smaller than that of  $\text{H}^+$ , which results in 25 % fewer  $^{23}\text{Na}$  spins being magnetized; and  $\text{Na}^+$  has a spin of  $3/2$ , which causes electrostatic field sensitivity and fast  $T_2^*$  signal decay. Thus, the total  $\text{Na}^+$  signal available on MRI in human tissue is only about  $1/22,000^{\text{th}}$  the size of the  $\text{H}^+$  signal (274). High static magnetic fields are commonly used with  $\text{Na}^+$  imaging to improve the signal to noise ratio. Moving to higher MRI field strengths, such as 7 Tesla, further increases the signal-to-noise, enabling higher spatial resolutions in faster acquisition times, which improves the probing of the tumour  $\text{Na}^+$  microenvironment (193).

Interest in performing  $^{23}\text{Na}$  MRI dates back as far as the 1980s. It was postulated that  $^{23}\text{Na}$  MRI would allow for superior contrast in distinguishing features of brain tumours such as oedema, necrosis and non-necrotic tumour, compared to conventional proton spin density imaging (275). Initial studies focused on healthy volunteers and animal models of stroke and myocardial infarction (275, 276), followed by the first images of brain tumour patients (277).

A set of publications followed (278-280) after which the focus turned to spectroscopic and animal model studies and small investigative human studies (190, 281). It was shown that  $^{23}\text{Na}$  MRI reliably revealed brain tumour lesions, albeit no correlation to histology or grading (190). Imaging developments in pulse sequence design and quantification methods were subsequently made to overcome shortcomings of previous studies, namely to increase spatial resolution and to capture the  $\text{Na}^+$  signal in its entirety by using ultra-short echo time imaging (282-284). Further detailed  $^{23}\text{Na}$  MRI studies followed in human brain and breast tumours (4, 9, 285). More recent advances in sodium imaging have shown that  $^{23}\text{Na}$  MRI can both predict IDH status (197) and show early response to pre-clinical therapeutic interventions (198). Furthermore, relaxation-weighted  $^{23}\text{Na}$  MRI has now been shown to differentiate between brain tumours of grades I-III and grade IV when spin-weighted  $^{23}\text{Na}$  MRI was unable to do so (286).

## Figure legends

**Figure 1.** Accumulation of  $\text{Na}^+$  in tumours. Converse to the reported decrease in  $\text{pO}_2$  and pH, many tumours (such as breast cancer) exhibit elevated  $[\text{Na}^+]$  (9). This elevated tumour  $[\text{Na}^+]$  may be due to increases in the extracellular volume fraction relative to the intracellular volume fraction, or due to increases in the  $[\text{Na}^+]$  concentration within either compartment. Moreover, tumour  $[\text{Na}^+]$  is likely influenced by the heterogeneity of the tumour microenvironment. Factors that could increase the extracellular volume fraction (interstitial volume) include increases in colloidal and interstitial pressure (13) due to vasculature permeabilisation, blood plasma protein release and the formation of fibrin (11, 12), and cancer cell death as a result of targeted chemotherapy or poor vascularisation within the necrotic tumour core. Moreover,  $\text{Na}^+$  binding to protein sequestered within the desmoplastic tumour microenvironment (e.g. glycosaminoglycans) could contribute to an increase in the extracellular  $\text{Na}^+$  content (287). Alternatively, the increased cellularity observed within poorly vascularised tumours (193) suggests that increases in intracellular volume fraction can contribute to elevated tumour  $[\text{Na}^+]$ ; indeed,  $[\text{Na}^+]_i$  has been reported to be elevated in cultured cancer cells (20, 22, 23), potentially due to altered transporter expression (Table 1).

**Figure 2.** Cellular  $\text{Na}^+$  import and export mechanisms. Cells exhibit a diverse repertoire of  $\text{Na}^+$  channels and transporters, many of which exhibit altered expression in cancer (Table 1) and are being explored as potential therapeutic targets (Table 2). The activity and conductance of these channels is regulated by  $[\text{Na}^+]_i$ ,  $[\text{Na}^+]_e$ , membrane potential and auxiliary regulatory proteins. Channels that facilitate  $\text{Na}^+$  influx include voltage gated  $\text{Na}^+$  channels (VGSC), epithelial  $\text{Na}^+$  channels (ENaC), acid-sensing channels (ASIC), glutamate-activated N-methyl-D-aspartate receptors (NMDA), ATP-activated P2X purinoceptor 7 (P2X<sub>7</sub>) and the G protein-coupled  $\text{Na}^+$  leak channel, non-selective (NALCN). The inward  $\text{Na}^+$  gradient and a hyperpolarised membrane potential are maintained by the ATP-driven  $\text{Na}^+/\text{K}^+$  ATPase.  $\text{Na}^+$  influx is also linked to the transport of numerous other ions

and substrates, namely  $H^+$  efflux ( $Na^+/H^+$  exchanger 1, NHE1),  $Cl^-$  and  $K^+$  influx ( $Na^+-K^+-Cl^-$  cotransporter, NKCC), cytosolic and mitochondrial  $Ca^{2+}$  efflux ( $Na^+/Ca^{2+}$  exchanger, NCX and mitochondrial  $Na^+-Ca^{2+}(Li^+)$  exchanger, NCLX, respectively) glucose uptake (sodium-glucose linked transporter, SGLT) and amino acid (AA) uptake.  $Na^+/H^+$  exchangers (NHE) are also present on both mitochondria and lysosomes, the latter of which achieve  $Na^+$  efflux into the cytosol via two-pore channels (TPC) and transient receptor potential mucolipin (TRPML) channels.

**Figure 3.** Physiological consequences of  $Na^+$  accumulation within tumours. Dashed arrows indicate putative mechanisms which remain to be fully characterised. Red arrows indicate denote movement/conversion of metabolites. *A: Elevated  $[Na^+]$  is linked to alterations in tumour metabolism and pH regulation.* Elevations in  $[Na^+]_i$  activate the  $Na^+/K^+$  ATPase, thereby raising ATP demand and driving a high glycolytic rate (34). To maintain a high  $pH_i$ , the resulting  $H^+$  is rapidly extruded by NHE, which is driven by the inward  $Na^+$  gradient. Increased  $[Na^+]_i$  could also facilitate depletion of mitochondrial  $Ca^{2+}$  ( $[Ca^{2+}]_m$ ) via NCLX, thereby altering mitochondrial metabolism. Conversely, changes to the  $Na^+$  gradient across the plasma membrane will likely alter the driving force for transporters importing key metabolic substrates such as glutamine (SLC1A5) and glucose (SGLT), thereby influencing anabolic and anapleurotic processes. *B: Elevated tumour  $Na^+$  and membrane potential ( $V_m$ ).*  $V_m$  is generated by the electrogenic  $Na^+/K^+$  ATPase;  $Na^+$  influx via VGSC/ENaC/ASICs results in a depolarised membrane potential ( $V_m$ ) in cancer cells. A depolarised  $V_m$  can lead to the activation of proliferative signalling cascades (such as KRas), cytoskeletal reorganisation facilitating migration, and accelerates the cell cycle. Conversely, most healthy differentiated cells exhibit a more hyperpolarised  $V_m$  that tightly controls cell cycle progression. *C: Cell volume regulation by  $Na^+$ -linked transport mechanisms.* Elevations in tumour  $[Na^+]$  are linked to changes in cell volume regulation. NKCC sequentially facilitates the accumulation of intracellular  $Cl^-$ ,  $H_2O$  uptake (aquaporins, AQP, and osmosis) and cell swelling (288). Conversely,  $K^+-Cl^-$  cotransporters (KCC) mediate  $Cl^-$  efflux, promoting  $H_2O$

exit from the cell (289). NHE1 activity results in a net osmotic gain due to  $\text{Na}^+$  influx; the osmolar contribution of intracellular  $\text{H}^+$  ions is compensated due to the dissociation of intracellular buffers. The resulting increase in  $\text{pH}_i$  and  $[\text{HCO}_3^-]_i$  drives  $\text{Cl}^-$  import via anion exchangers (AE), leading to  $\text{H}_2\text{O}$  uptake and cell swelling (290). NHE1 can also operate to in reverse mode to resist compressive forces (118).

**Figure 4.** Effect of elevated  $\text{Na}^+$  on cancer progression and the tumour microenvironment.

Dashed arrows indicate putative mechanisms which remain to be fully characterised. *A:*

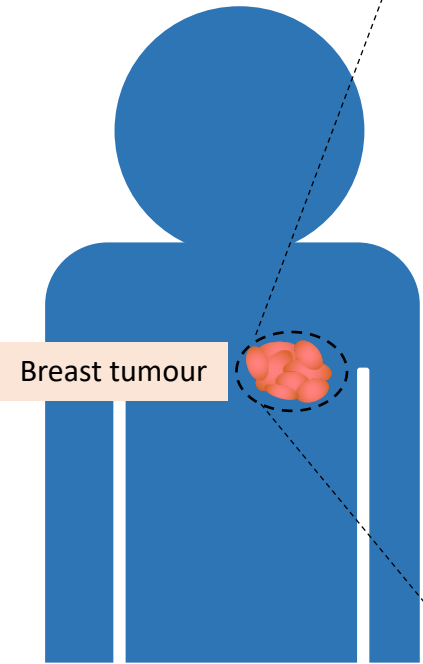
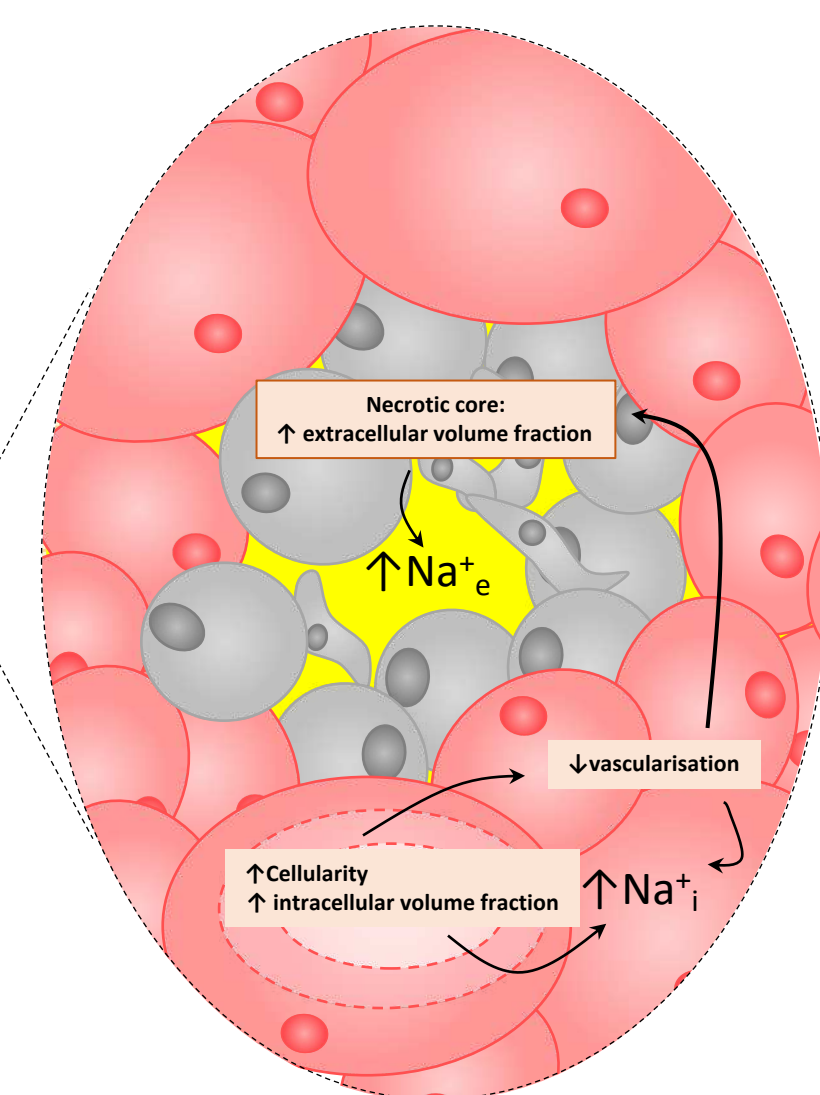
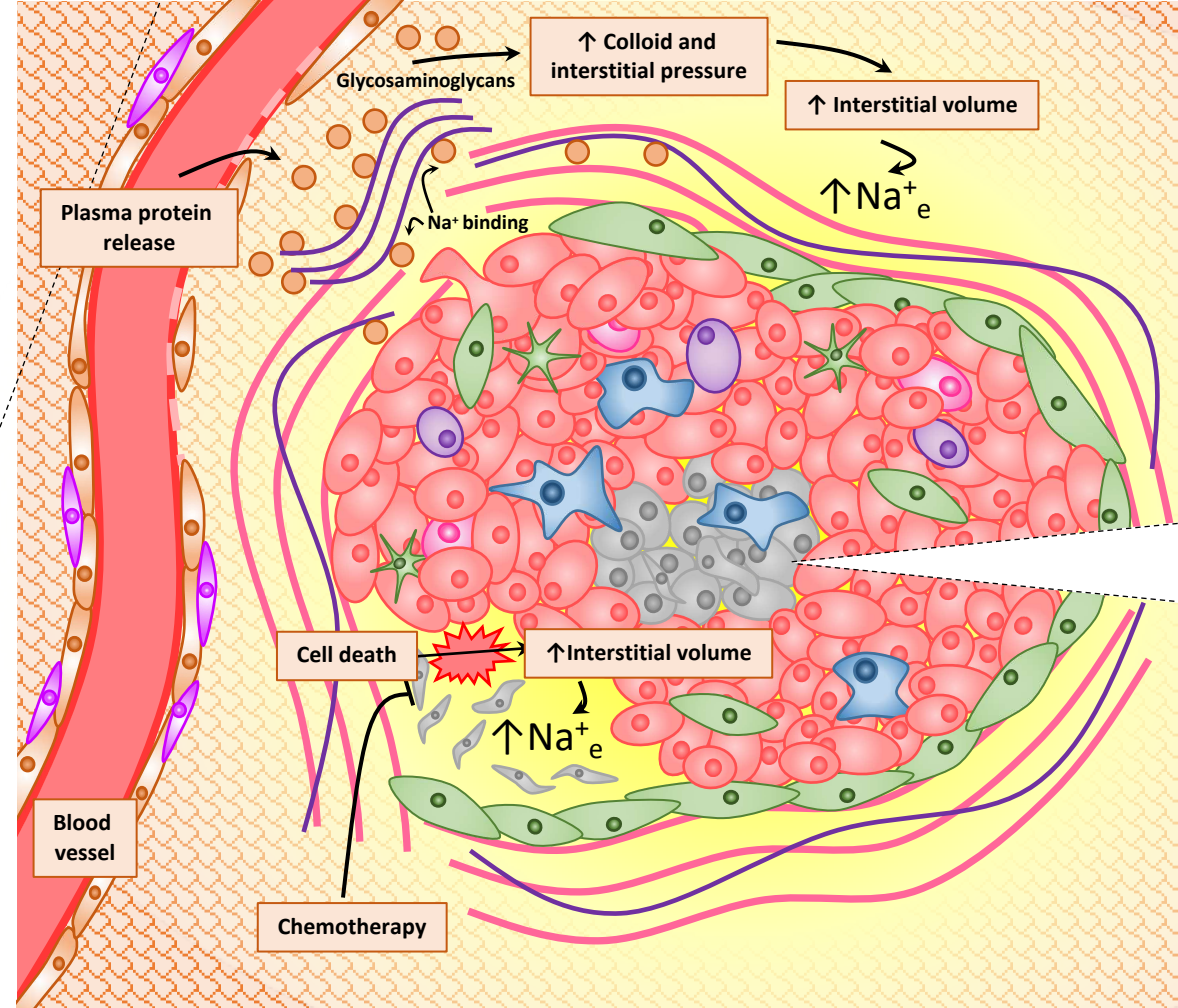
*Elevated tumour  $\text{Na}^+$  and migration/invasion.* VGSCs have been correlated with activation of a proinvasive gene transcription network upregulating Wnt, MAPK and  $\text{Ca}^{2+}$  signalling (161, 164). NHE1 localises to the leading edge of invading cells (136); VGSC colocalisation with NHE1 within caveolae leads to activation of NHE1, acidification of the extracellular environment and digestion of the extracellular matrix by cathepsins and matrix metalloproteinases (120, 142, 152). Interestingly, the  $\beta$  subunit of VGSCs acts as an adhesion molecule that interacts with the extracellular matrix to regulate migration and invasion (291). Extracellular acidification can also activate ASIC and ENaC channels (58, 59), leading to further increases in  $[\text{Na}^+]_i$ .  $\text{Na}^+$ -linked  $\text{Ca}^{2+}$  influx via reverse-mode NCX action has been linked to cancer cell motility via  $\text{Ca}^{2+}$  signalling-activated TGF- $\beta$  signalling (151). NKCC regulates cell swelling required for migratory behaviour by facilitating  $[\text{Cl}^-]_i$  accumulation and  $\text{H}_2\text{O}$  uptake via osmosis and aquaporins (288). NKCC also acts as a scaffold for cofilin within invadopodia, which facilitates cytoskeletal remodelling (292). *B:* *Elevated  $[\text{Na}^+]_e$  and cancer cell proliferation.* The inward  $\text{Na}^+$  gradient drives the uptake of anabolic substrates such as glucose and glutamine (SGLT and SLC1A5), respectively (44-46); altered tumour  $[\text{Na}^+]$  might regulate the uptake of these substrates. Via glycolysis/pentose phosphate pathway (PPP) and glutaminolysis, glucose and glutamine are utilised as substrates for redox homeostasis, biosynthesis, and cell proliferation (i.e. reducing equivalents, nucleotides, and fatty acids) (293). SLC1A5 also regulates mTORC1, a key regulator of protein translation and cell growth (254). Moreover, salt inducible kinase 3

is activated by elevated  $[\text{Na}^+]_e$ , promoting G1/S phase transition (129), and  $[\text{Cl}^-]$  accumulation due to upregulated NKCC activity can promote cell cycle progression (48, 117, 130). Elevated  $[\text{Na}^+]_e$  leads to DNA breaks with significant implications for oncogenic mutations/tumour suppressor silencing (132), and a high osmolality and VGSC activity also activates the MAPK signalling cascade, potentially via Rac1 activation (127, 128). C:

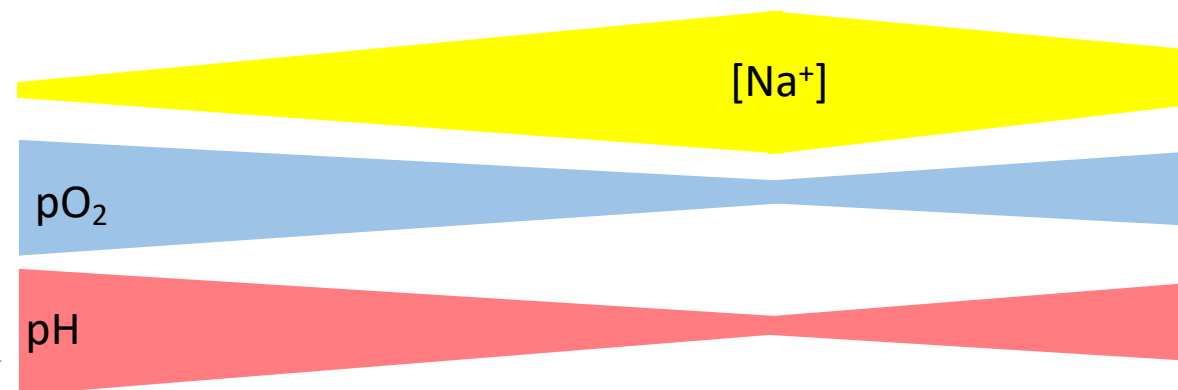
*Elevated  $\text{Na}^+$  and osmolality drives inflammation in the tumour microenvironment.* Increased  $[\text{Na}^+]_e$  and osmolality promote proliferative and antiapoptotic signalling in tumour associated macrophages (171) and by increasing the production of proinflammatory cytokines by local endothelial cells and Th-17 helper cells (177). Together these factors also promote the further recruitment of proinflammatory immune cells (171). These factors lead to extracellular matrix breakdown, neovascularisation and tumour remodelling, thereby promoting tumour progression and metastasis (129, 179, 187, 294).

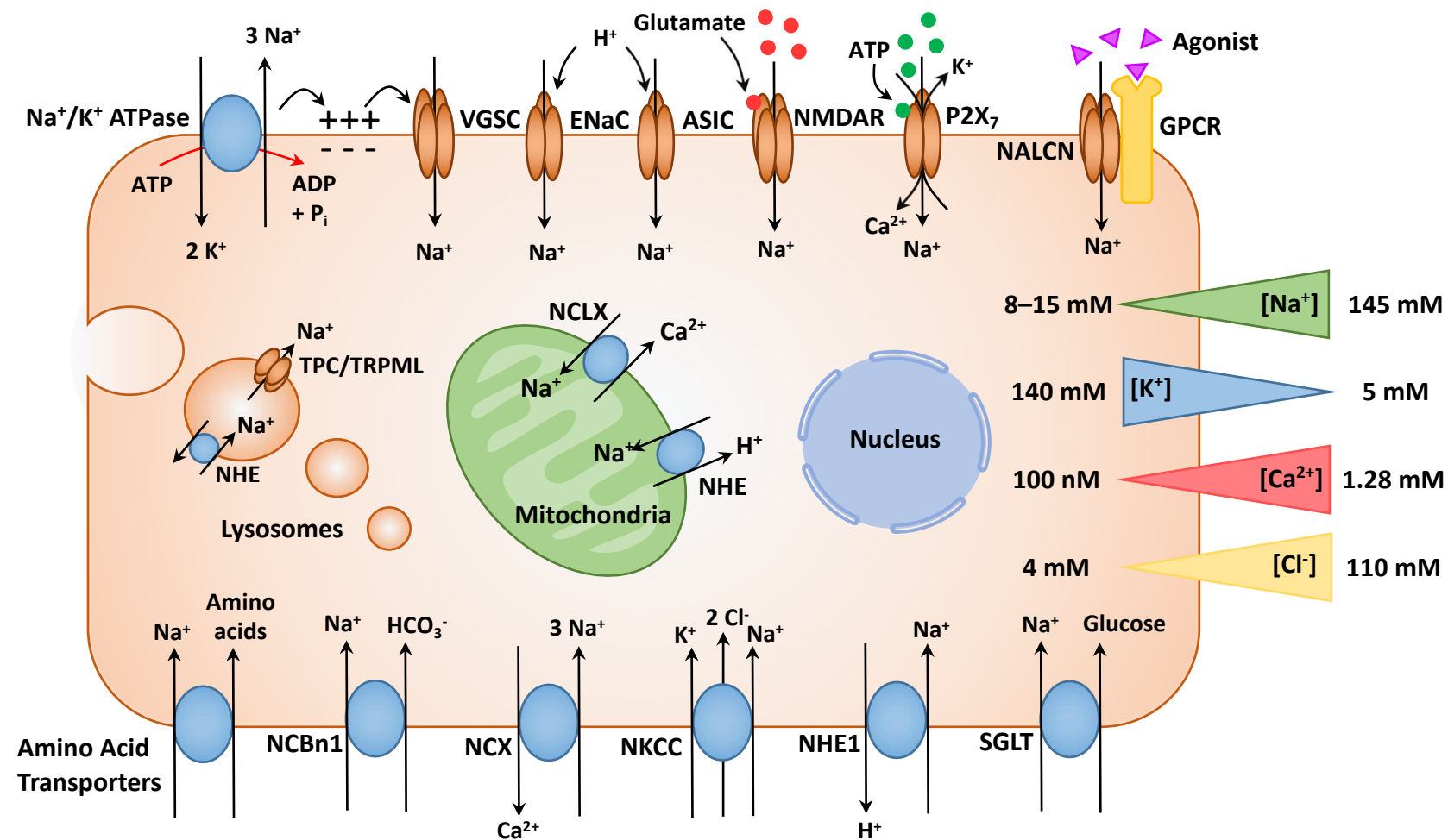


- Malignant cancer cell
- Necrotic cancer cell
- Dendritic cell
- Macrophage
- T-lymphocyte
- CAF
- Cancer stem cell
- Endothelial cell
- Collagen
- Fibrin
- Pericyte

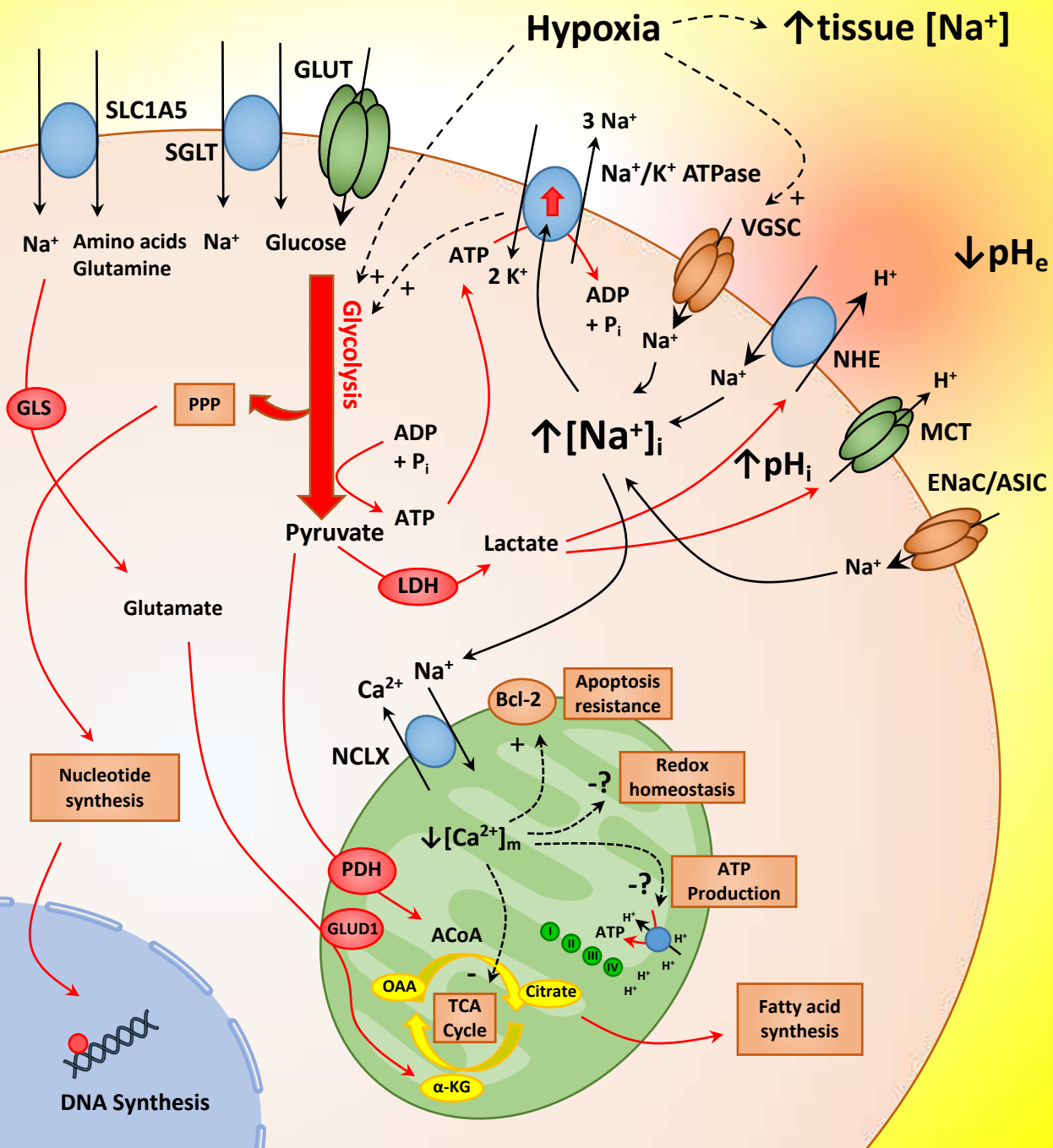


Breast tumour

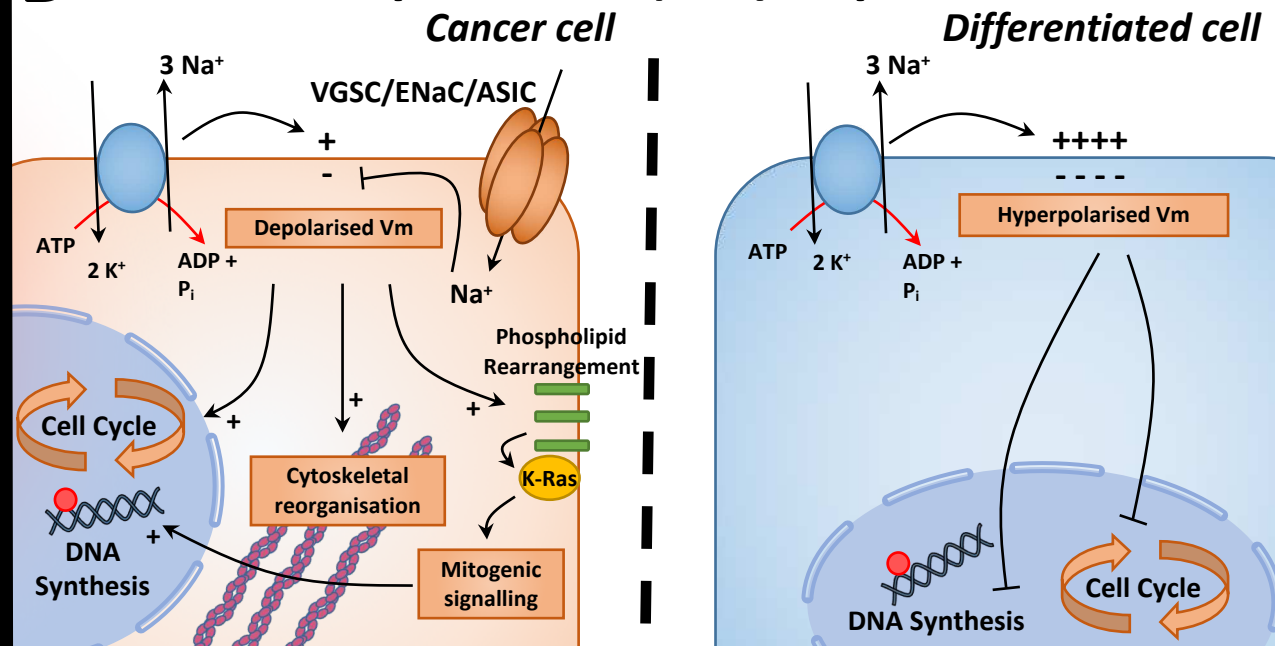




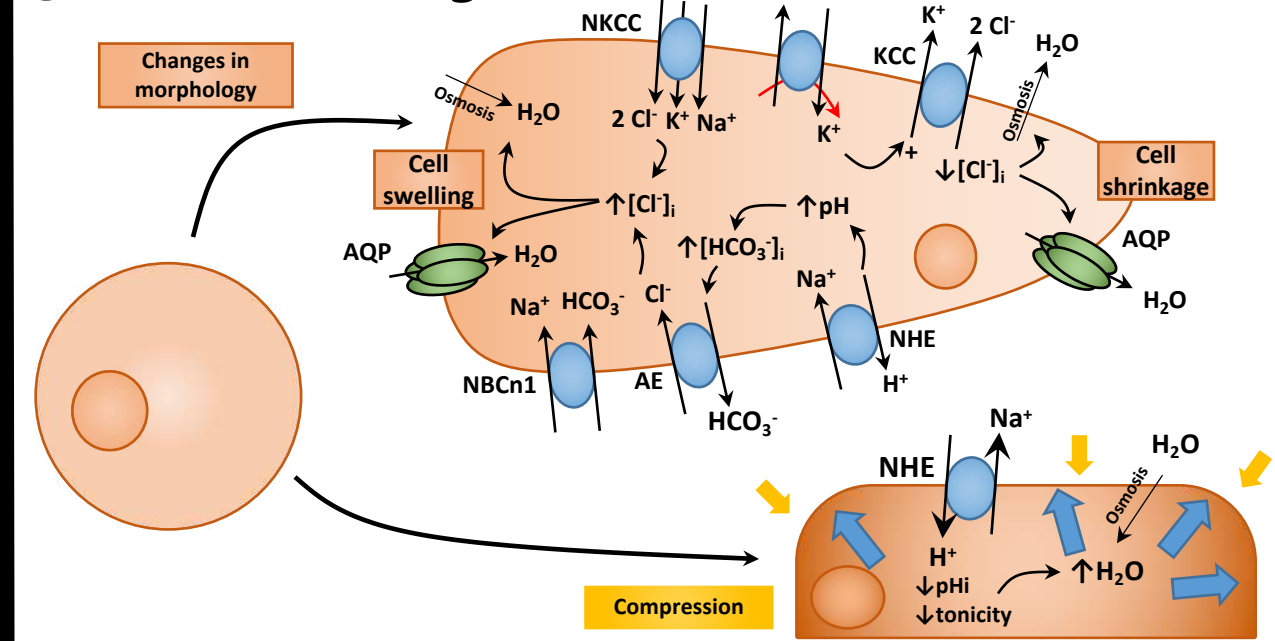
# A Altered metabolism



# B Membrane potential (V<sub>m</sub>) depolarisation

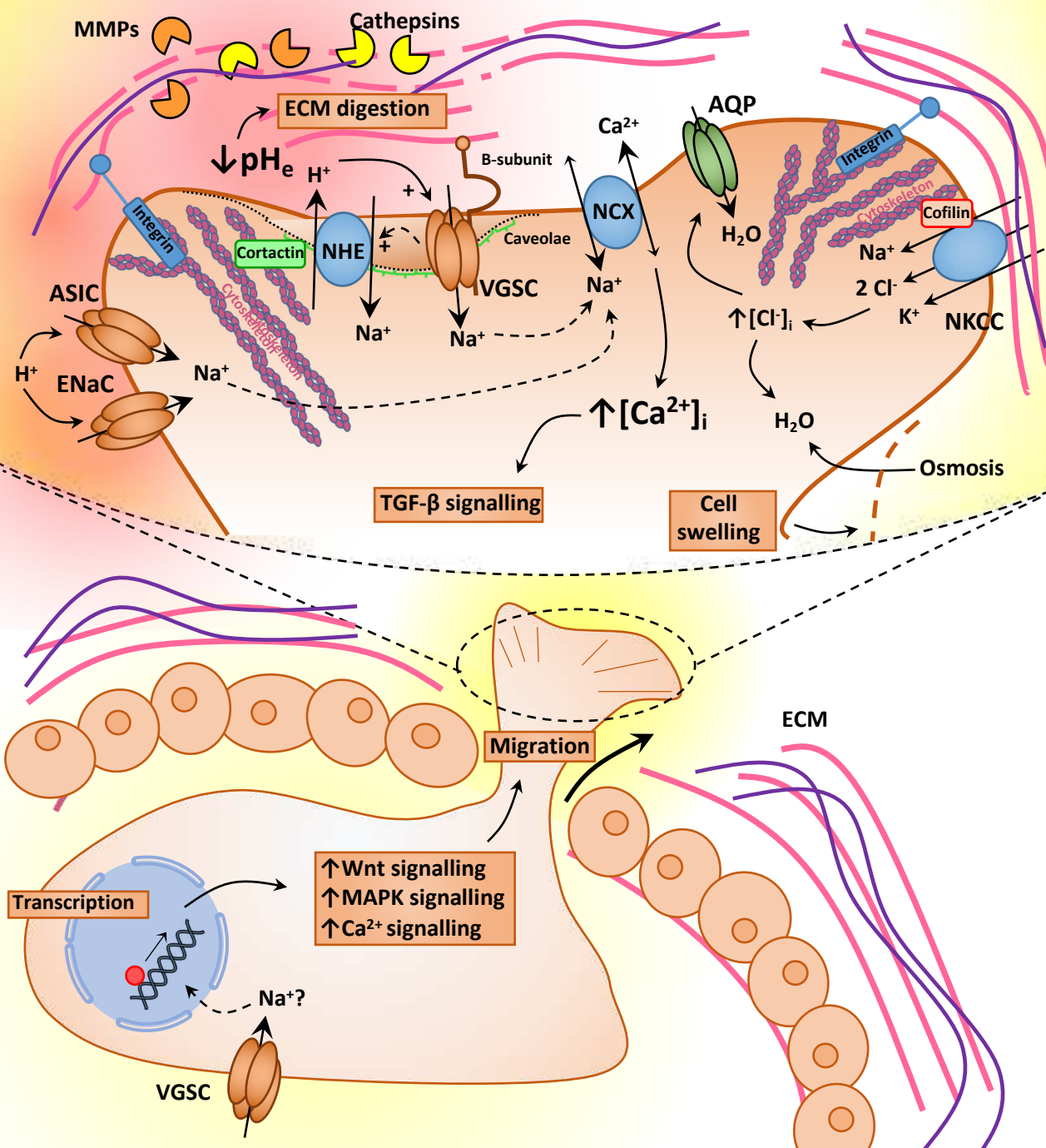


# C Cell volume regulation

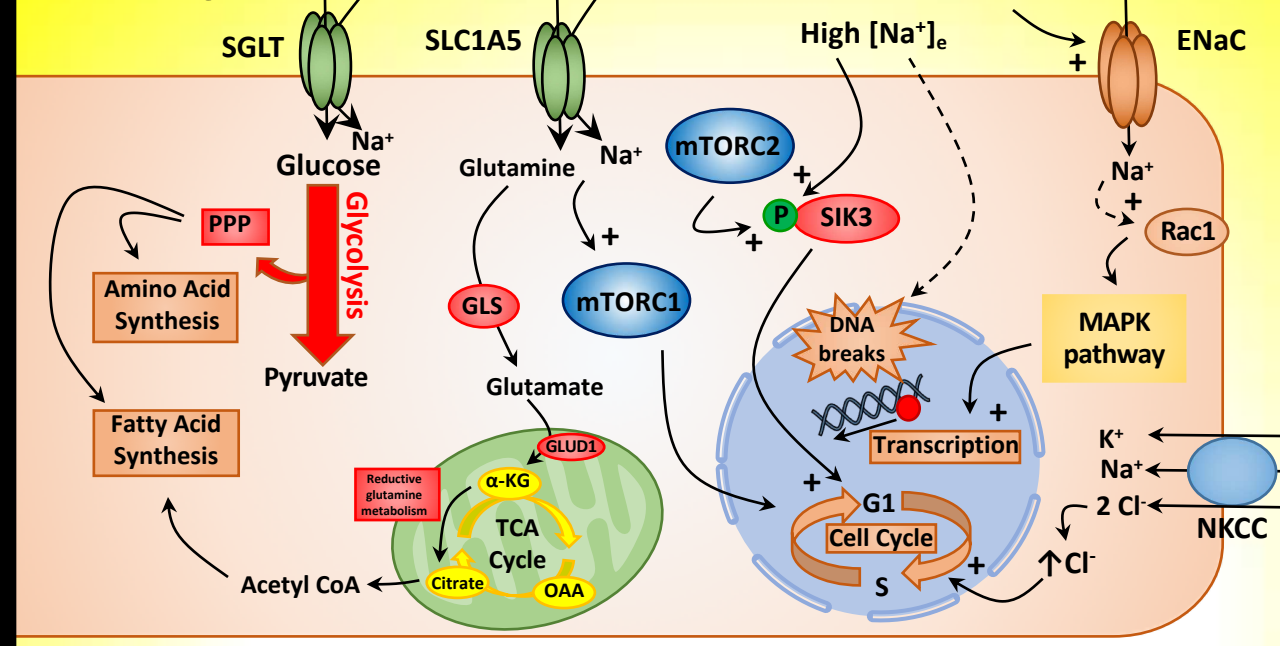




# A Invasion



# B Proliferation



# C Tumour inflammation/microenvironment

